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WO 02/102235

PCT/US02/19297

METHODS OF DIAGNOSIS OF OVARIAN CANCER, COMPOSITIONS AND METHODS OF SCREENING FOR MODULATORS OF OVARIAN CANCER

CROSS-REFERENCES TO RELATED APPLICATIONS.

60/350,666, filed November 13, 2001; and USSN 60/372,246, filed April 12, 2002, each of 60/315,287, filed August 27, 2001; USSN 60/317,544, filed September 5, 2001; USSN This application is related to USSN 60/299,234, filed June 18, 2001; USSN which is incorporated herein by reference for all purposes. S

#### FIELD OF THE INVENTION

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The invention relates to the identification of nucleic acid and protein expression profiles and nucleic acids, products, and antibodies thereto that are involved in ovarian prognosis, and therapy of ovarian cancer. The invention further relates to methods for cancer; and to the use of such expression profiles and compositions in the diagnosis, identifying and using agents and/or targets that inhibit ovarian cancer.

## BACKGROUND OF THE INVENTION

Society predicts that there will be about 23,100 new cases of ovarian cancer in this country in Ovarian cancer is the sixth most common cancer in women, accounting for 6% of all emale cancers. It ranks fifth as the cause of cancer death in women. The American Cancer cancers cannot be detected early in their development, they account for a disproportionate number of fatal cancers, being responsible for almost half the deaths from cancer of the he year 2000 and about 14,000 women will die of the disease. Because many ovarian emale genital tract; more deaths than any other reproductive organ cancer. 2

Most patients with epithelial ovarian cancer, the predominant form, are asymptomatic 30%-90%. See, Parker, et. al.. (1997) "Cancer Statistics, 1997" CA Cancer J. Clin., 47:5-27. minority of patients discovered with early-stage disease have a five-year survival rate of n early-stage disease and usually present with stage III or IV disease. Their five-year survival is less than 25%, with lower survival among African-American women. The 22

170. Risk factors include familial cancer syndromes (risk of up to 82% by age 70 in women In the absence of a family history of ovarian cancer, lifetime risk of ovarian cancer is 3

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(57) Abstract: Described herein are genes whose expression are up-regulated or down-regulated in ovarian cancer. Related methods and compositions that can be used for diagnosts and treatment of ovarian cancer are disclosed. Also described herein are methods

that can be used to identify modulators of ovarian cancer.

(54) THE: METHODS OF DIAGNOSIS OF OVARIAN CANCER, COMPOSITIONS AND METHODS OF SCREENING FOR

MODULATORS OF OVARIAN CANCER

WO 02/102235

with hereditary breast/ovarian syndrome); family history (1.4% lifetime risk with no affected relatives, 5% with one affected relative, 7% with two affected relatives; Kerlikowske, et.al. (1992) <u>Obstet. Gynecol.</u> 80:700-707); nulliparity; advancing age; obesity; personal history of breast, endometrial, or colorectal cancer; fewer pregnancies; or older age (>35 years) at first pregnancy. However, 95% of all ovarian cancers occur in women without risk factors. Use of hormonal contraceptives, oophorectomy, and tubal sterilization reduce risk of ovarian cancer (Kerlikowske, et. al. (1992) <u>Obstet. Gynecol.</u> 80:700-707; Grimes (1992) <u>Am J. Obstet. Gynecol.</u> 166:1950-1954; Hankinson, et. al. (1993) <u>IAMA</u> 270:2813-2818); however, even hilateral oophorectomy may not be completely effective in preventing ovarian cancer.

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Treatment of ovarian cancer consists largely of surgical oophorectomy, anti-hormone therapy, and/or chemotherapy. Although many ovarian cancer patients are effectively treated, the current therapics can all induce scrious side effects which diminish quality of life. Deciding on a particular course of treatment is typically based on a variety of prognostic parameters and markers (Fitzgibbons, et al. (2000) <u>Arch. Pathol. Lab. Med.</u> 124:966-978; Hamilton and Piccart (2000) <u>Ann. Oncol.</u> 11:647-663), including genetic predisposition markers BRCA-1 and BRCA-2 (Robson (2000) <u>I. Clin. Oncol.</u> 18:113sup-118sup).

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The identification of novel therapeutic targets and diagnostic markers is essential for improving the current treatment of ovarian cancer patients. Recent advances in molecular medicine have increased the interest in tumor-specific cell surface antigens that could serve as targets for various immunotherapeutic or small molecule strategies. Antigens suitable for immunotherapeutic strategies should be highly expressed in cancer tissues and ideally not expressed in normal adult tissues. Expression in tissues that are dispensable for life, however, may be tolerated. Examples of such antigens include Her2/neu and the B-cell antigen CD20. Humanized monoclonal antibodies directed to Her2/neu (Herceptin®/trastuzumab) are currently in use for the treatment of metastatic breast cancer. Ross and Fletcher (1998) <u>Stem Cells</u> 16:413-428. Similarly, anti-CD20 monoclonal antibodies (Rituxin®/rituximab) are used to effectively treat non-Hodgkin's lymphoma. Maloney, et al. (1997) <u>Blood</u> 90:2188-2195; Leget and Czuczman (1998) <u>Curr. Opin. Oncol.</u>

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Potential immunotherapeutic targets have been identified for ovarian cancer. One such target is polymorphic epithelial mucin (MUC1). MUC1 is a transmembrane protein, present at the apical surface of glandular epithelial cells. It is often overexpressed in ovarian cancer, and typically exhibits an altered glycosylation pattern, resulting in an antigenically

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WO 02/102235 PCT/US02/19297

distinct molecule, and is in early clinical trials as a vaccine target. Gilewski, et al. (2000) Clin. Cancer Res. 6:1693-1701; Scholl, et al. (2000) <u>I. Immunother.</u> 23:570-580. The tumor-expressed protein is often cleaved into the circulation, where it is detectable as the tumor marker, CA 15-3. See, e.g., Bon, et al. (1997) <u>Clin. Chem.</u> 43:585-593. However, many patients have tumors that express neither HER2 nor MUC-1; therefore, it is clear that other

Mutations in both BRCA1 and BRCA2 are associated with increased susceptibility to ovarian cancer. Mutations in BRCA1 occur in approximately 5 percent (95 percent confidence interval, 3 to 8 percent) of women in whom ovarian cancer is diagnosed before

targets need to be identified to manage localized and metastatic disease.

10 the age of 70 years. See Stratţon, et al. (1997) N.E.J. Med. 336:1125-1130. And, in BRCA1 gene carriers, the risk for developing ovarian cancer is .63. See Easton (1995) Am. J. Hum. Genet. 56:267-xxx; and Elit (2001) Can. Fam. Physician 47:778-84.

Other biochemical markers such as CA125 have been reported to be associated with ovarian cancer, but they are not absolute indicators of disease. Although roughly 85% of women with clinically apparent ovarian cancer have increased levels of CA125, CA125 is also increased during the first trimester of pregnancy, during menstruation, in the presence of non-cancerous illnesses, and in cancers of other sites.

While industry and academia have identified novel gene sequences, there has not been an equal effort exerted to identify the function of those novel sequences. The elucidation of a role for novel proteins and compounds in disease states for identification of therapeutic targets and diagnostic markers is essential for improving the current treatment of ovarian cancer patients. Accordingly, provided herein are molecular targets for therapeutic intervention in ovarian and other cancers. Additionally, provided herein are methods that can be used in diagnosis and prognosis of ovarian cancer. Further provided are methods that can

25 be used to screen candidate bioactive agents for the ability to modulate ovarian cancer.

## SUMMARY OF THE INVENTION

The present invention therefore provides nucleotide sequences of genes that are upand down-regulated in ovarian cancer cells. Such genes are useful for diagnostic purposes,
and also as targets for screening for therapeutic compounds that modulate ovarian cancer,
such as hormones or antibodies. The methods of detecting nucleic acids of the invention or
their encoded proteins can be used for many purposes, e.g., early detection of ovarian
cancers, monitoring and early detection of relapse following treatment, moniforing response
to therapy, selecting patients for postoperative chemotherapy or radiation therapy, selecting
therapy, determining tumor prognosis, treatment, or response to treatment (of primary or
metastatic tumors), and early detection of pre-cancerous lesions. Other aspects of the
invention will become apparent to the skilled artisan by the following description of the

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In one aspect, the present invention provides a method of detecting an ovarian cancer-associated transcript in a cell from a patient, the method comprising contacting a biological sample from the patient with a polynucleotide that selectively hybridizes to a sequence at least 80% identical to a sequence as shown in Tables 1-26.

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In one embodiment, the present invention provides a method of determining the level of an ovarian cancer associated transcript in a cell from a patient.

In one embodiment, the present invention provides a method of detecting an ovarian cancer-associated transcript in a cell from a patient, the method comprising contacting a biological sample from the patient with a polynucleotide that selectively hybridizes to a sequence at least 80% identical to a sequence as shown in Tables 1-26.

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In one embodiment, the polynucleotide selectively hybridizes to a sequence at least 95% identical to a sequence as shown in Tables 1-26.

In one embodiment, the biological sample is a tissue sample. In another embodiment, the biological sample comprises isolated nucleic acids, e.g., mRNA.

In one embodiment, the polynucleotide is labeled, e.g., with a fluorescent label. In one embodiment, the polynucleotide is immobilized on a solid surface.

In one embodiment, the patient is undergoing a therapeutic regimen to treat ovarian

cancer. In another embodiment, the patient is suspected of having metastatic ovarian cancer. In one embodiment, the patient is a human.

8

In one embodiment, the ovarian cancer associated transcript is mRNA.

In one embodiment, the method further comprises the step of amplifying nucleic acids

WO 02/102235 PCT/US02/19297

before the step of contacting the biological sample with the polynucleotide.

In another aspect, the present invention provides a method of monitoring the efficacy of a therapeutic treatment of ovarian cancer, the method comprising the steps of: (i) providing a biological sample from a patient undergoing the therapeutic treatment; and (ii) determining the level of an ovarian cancer-associated transcript in the biological sample by contacting the biological sample with a polymucleotide that selectively hybridizes to a sequence at least 80% identical to a sequence as shown in Tables 1-26, thereby monitoring the efficacy of the therapy. In a further embodiment, the patient has metastatic ovarian cancer. In a further embodiment, the patient has a drug resistant form of ovarian cancer.

In one embodiment, the method further comprises the step of: (iii) comparing the level of the ovarian cancer-associated transcript to a level of the ovarian cancer-associated transcript in a biological sample from the patient prior to, or earlier in, the therapeutic

Additionally, provided herein is a method of evaluating the effect of a candidate ovarian cancer drug comprising administering the drug to a patient and removing a cell sample from the patient. The expression profile of the cell is then determined. This method may further comprise comparing the expression profile to an expression profile of a healthy individual. In a preferred embodiment, said expression profile includes a gene of Tables 1-

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In one aspect, the present invention provides an isolated nucleic acid molecule consisting of a polynucleotide sequence as shown in Tables 1-26.

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In one embodiment, an expression vector or cell comprises the isolated nucleic acid.

In one aspect, the present invention provides an isolated polypeptide which is encoded by a nucleic acid molecule having polynucleotide sequence as shown in Tables 1-26.

In another aspect, the present invention provides an antibody that specifically binds to an isolated polypeptide which is encoded by a nucleic acid molecule having polynucleotide sequence as shown in Tables 1-26.

In one embodiment, the antibody is conjugated to an effector component, e.g., a fluorescent label, a radioisotope or a cytotoxic chemical.

30 In one embodiment, the antibody is an antibody fragment. In another embodiment, the antibody is humanized.

In one aspect, the present invention provides a method of detecting an ovarian cancer cell in a biological sample from a patient, the method comprising contacting the biological

WO 02/102235

PCT/US02/19297

sample with an antibody as described herein.

In another aspect, the present invention provides a method of detecting antibodies specific to ovarian cancer in a patient, the method comprising contacting a biological sample from the patient with a polypeptide encoded by a nucleic acid comprising a sequence from Tahles 1.26

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In another aspect, the present invention provides a method for identifying a compound that modulates an ovarian cancer-associated polypeptide, the method comprising the steps of:

(i) contacting the compound with an ovarian cancer-associated polypeptide, the polypeptide encoded by a polymucleotide that selectively hybridizes to a sequence at least 80% identical to a sequence as shown in Tables 1-26; and (ii) determining the functional effect of the compound upon the polypeptide.

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In one embodiment, the functional effect is a physical effect, an enzymatic effect, or a chemical effect.

In one embodiment, the polypeptide is expressed in a cukaryotic host cell or cell membrane. In another embodiment, the polypeptide is recombinant.

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In one embodiment, the functional effect is determined by measuring ligand binding to the polypeptide.

In another aspect, the present invention provides a method of inhibiting proliferation of an ovarian cancer-associated cell to treat ovarian cancer in a patient, the method comprising the step of administering to the subject a therapeutically effective amount of a compound identified as described herein.

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In one embodiment, the compound is an antibody.

In another aspect, the present invention provides a drug screening assay comprising the steps of: (i) administering a test compound to a mammal having ovarian cancer or to a cell sample isolated from; (ii) comparing the level of gene expression of a polynucleotide that selectively hybridizes to a sequence at least 80% identical to a sequence as shown in Tables 1-26 in a treated cell or mammal with the level of gene expression of the polynucleotide in a control cell sample or mammal, wherein a test compound that modulates the level of expression of the polynucleotide is a candidate for the treatment of ovarian cancer.

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In one embodiment, the control is a mammal with ovarian cancer or a cell sample that has not been treated with the test compound. In another embodiment, the control is a normal cell or mammal, or is non-malignant tissue.

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In one embodiment, the test compound is administered in varying amounts or

concentrations. In another embodiment, the test compound is administered for varying time periods. In another embodiment, the comparison can occur after addition or removal of the drug candidate.

In one embodiment, the levels of a plurality of polynucleotides that selectively

hybridize to a sequence at least 80% identical to a sequence as shown in Tables 1-26 are individually compared to their respective levels in a control cell sample or manmal. In a preferred embodiment the plurality of polynucleotides is from three to ten.

In another aspect, the present invention provides a method for treating a mammal having ovarian cancer comprising administering a compound identified by the assay

10 described herein.

In another aspect, the present invention provides a pharmaceutical composition for treating a mammal having ovarian cancer, the composition comprising a compound identified by the assay described herein and a physiologically acceptable excipient.

In one aspect, the present invention provides a method of screening drug candidates by providing a cell expressing a gene that is up- and down-regulated as in an ovarian cancer. In one embodiment, a gene is selected from Tables 1-26. The method further includes adding a drug candidate to the cell and determining the effect of the drug candidate on the expression of the expression profile gene.

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In one embodiment, the method of screening drug candidates includes comparing the level of expression in the absence of the drug candidate to the level of expression in the presence of the drug candidate, wherein the concentration of the drug candidate can vary when present, and wherein the comparison can occur after addition or removal of the drug candidate. In a preferred embodiment, the cell expresses at least two expression profile genes. The profile genes may show an increase or decrease.

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Also provided is a method of evaluating the effect of a candidate ovarian cancer drug comprising administering the drug to a transgenic animal expressing or over-expressing the ovarian cancer modulatory, protein, or an animal lacking the ovarian cancer modulatory protein, for example as a result of a gene knockout.

Moreover, provided herein is a biochip comprising one or more nucleic acid segments of Tables 1-26, wherein the biochip comprises fewer than 1000 nucleic acid probes. Preferably, at least two nucleic acid segments are included. More preferably, at least three nucleic acid segments are included.

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Furthermore, a method of diagnosing a disorder associated with ovarian cancer is

first tissue type of a first individual, and comparing the distribution to the expression of the provided. The method comprises determining the expression of a gene of Tables 1-26 in a individual. A difference in the expression indicates that the first individual has a disorder gene from a second normal tissue type from the first individual or a second unaffected associated with ovarian cancer.

In a further embodiment, the biochip also includes a polynucleotide sequence of a gene that is not up- and down-regulated in ovarian cancer. In one embodiment a method for screening for a bioactive agent capable of interfering with the binding of an ovarian cancer modulating protein (ovarian cancer modulatory protein) or fragment thereof. In a preferred embodiment, the method comprises combining an ovarian or a fragment thereof and an antibody which binds to said ovarian cancer modulatory protein cancer modulatory protein or fragment thereof, a candidate bioactive agent and an antibody identified as an interfering agent. The interfering agent can be an agonist or an antagonist. which binds to said ovarian cancer modulatory protein or fragment thereof. The method fragment thereof and said antibody. Wherein there is a change in binding, an agent is further includes determining the binding of said ovarian cancer modulatory protein or Preferably, the agent inhibits ovarian cancer.

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another embodiment, the protein is encoded by a nucleic acid selected from those of Tables Also provided herein are methods of eliciting an immune response in an individual. In one embodiment a method provided herein comprises administering to an individual a composition comprising an ovarian cancer modulating protein, or a fragment thereof. In

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thereof, and a pharmaceutically acceptable carrier. In another embodiment, said composition Further provided herein are compositions capable of eliciting an immune response in cancer modulating protein, preferably encoded by a nucleic acid of Table 1-26 or a fragment protein, preferably selected from the nucleic acids of Tables 1-26, and a pharmaccutically an individual. In one embodiment, a composition provided herein comprises an ovarian comprises a nucleic acid comprising a sequence encoding an ovarian cancer modulating acceptable carrier.

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fragment thereof, comprising contacting an agent specific for said protein with said protein in Also provided are methods of neutralizing the effect of an ovarian cancer protein, or a an amount sufficient to effect neutralization. In another embodiment, the protein is encoded by a nucleic acid selected from those of Tables 1-26. 3

WO 02/102235

PCT/US02/19297

cancer modulating protein conjugated to a therapeutic moiety. Such a therapeutic moiety can nethod comprises administering to a patient having ovarian cancer an antibody to an ovarian individual an inhibitor of an ovarian cancer modulating protein. In another embodiment, the In another aspect of the invention, a method of treating an individual for ovarian cancer is provided. In one embodiment, the method comprises administering to said be a cytotoxic agent or a radioisotope.

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PCT/US02/19297

## DETAILED DESCRIPTION OF THE INVENTION

methods for diagnosis and prognosis evaluation for ovarian cancer (OC), including metastatic ovarian carcinoma (e.g., epithelial (including malignant serous tumors, malignant mucinous dysgerminoma, and gonadoblastoma), and stromal carcinomas (e.g., granulosal stromal cell In accordance with the objects outlined above, the present invention provides novel ovarian cancer, as well as methods for screening for compositions which modulate ovarian cancer. Also provided are methods for treating ovarian cancer and related conditions, e.g., choriocarcinomas, polyembryomas, embryonal carcinoma, endodermal sinus tumor, tumors, and malignant endometrioid tumors), germ cell (including teratomas, tumors)), fallopian tube carcinoma, and peritoneal carcinoma.

Tables 1-26 also provide an exemplar accession number that provides a nucleotide sequence sequence of genes that exhibit increased or decreased expression in ovarian cancer samples. Tables 1-26 provide unigene cluster identification numbers for the nucleotide that is part of the unigene cluster.

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Definitions

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comprising an amino acid sequence encoded by a nucleotide sequence of or associated with a acid sequence that has greater than about 60% amino acid sequence identity, 65%, 70%, 75% 100, 200, 500, 1000, or more amino acid, to an amino acid sequence encoded by a nucleotide sequence identity, preferably over a region of over a region of at least about 25, 50, 100, 200, gene of Tables 1-26, and conservatively modified variants thereof; (3) specifically hybridize Tables 1-26; (2) bind to antibodies, e.g., polyclonal antibodies, raised against an immunogen 80%, 85%, 90%, preferably 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or greater alleles, mutants, and interspecies homologues that: (1) have a nucleotide sequence that has thereof of Tables 1-26 and conservatively modified variants thereof; or (4) have an amino amino sequence identity, preferably over a region of over a region of at least about 25, 50, The term "ovarian cancer protein" or "ovarian cancer polynucleotide" or "ovarian cancer-associated transcript" refers to nucleic acid and polypeptide polymorphic variants, 500, 1000, or more nucleotides, to a nucleotide sequence of or associated with a gene of greater than about 60% nucleotide sequence identity, 65%, 70%, 75%, 80%, 85%, 90%, under stringent hybridization conditions to a nucleic acid sequence, or the complement sequence of or associated with a gene of Tables 1-26. A polynucleotide or polypeptide preferably 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or greater nucleotide

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PCT/US02/19297 WO 02/102235

cancer polypeptide" and an "ovarian cancer polynucleotide," include both naturally occurring sequence is typically from a mammal including, but not limited to, primate, e.g., human; rodent, e.g., rat, mouse, harnster; cow, pig, horse, sheep, or other mammal. An "ovarian or recombinant forms.

- polypeptide or polynucleotide sequence, or a variant thereof, that contains all of the elements polynucleotide or polypeptide sequences. The "full length" may be prior to, or after, various A "full length" ovarian cancer protein or nucleic acid refers to an ovarian cancer normally contained in one or more naturally occurring, wild type ovarian cancer stages of post-translation processing or splicing, including alternative splicing.
- contains nucleic acids or polypeptides, e.g., of an ovarian cancer protein, polynucleotide or transcript. Such samples include, but are not limited to, tissue isolated from primates, e.g., numans, or rodents, e.g., mice, and rats. Biological samples may also include sections of tissues such as biopsy and autopsy samples, frozen sections taken for histologic purposes, 'Biological sample" as used herein is a sample of biological tissue or fluid that 2
- include explants and primary and/or transformed cell cultures derived from patient tissues. A mammal such as a primate e.g., chimpanzee or human; cow; dog; cat; a rodent, e.g., guinea blood, plasma, serum, sputum, stool, tears, mucus, hair, skin, etc. Biological samples also pig, rat, mouse; rabbit; or a bird; reptile; or fish. Livestock and domestic animals are of biological sample is typically obtained from a eukaryotic organism, most preferably a particular interest. 15 ឧ

solated by another person, at another time, and/or for another purpose), or by performing the methods of the invention in vivo. Archival tissues, having treatment or outcome history, will methods described in this invention. Most often, this will be done by removing a sample of cells from an animal, but can also be accomplished by using previously isolated cells (e.g., Providing a biological sample" means to obtain a biological sample for use in be particularly useful.

acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region, when compared and aligned for maximum correspondence over a comparison window or designated region) as (e.g., about 60% identity, preferably 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, The terms "identical" or percent "identity," in the context of two or more nucleic measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default

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at least about 25 amino acids or nucleotides in length, or more preferably over a region that is algorithms can account for gaps and the like. Preferably, identity exists over a region that is web site http://www.ncbi.nfm.nih.gov/BLAST/ or the like). Such sequences are then said to and/or additions, as well as those that have substitutions, as well as naturally occurring, e.g., parameters described below, or by manual alignment and visual inspection (see, e.g., NCBI polymorphic or allelic variants, and man-made variants. As described below, the preferred compliment of a test sequence. The definition also includes sequences that have deletions be "substantially identical." This definition also refers to, or may be applied to, the 50-100 amino acids or nucleotides in length.

program parameters can be used, or alternative parameters can be designated. The sequence which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Preferably, default comparison algorithm then calculates the percent sequence identities for the test sequences For sequence comparison, typically one sequence acts as a reference sequence, to relative to the reference sequence, based on the program parameters. 2

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A "comparison window", as used herein, includes reference to a segment of one of the 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, may be compared to a reference sequence of the same number of contiguous positions after 2.482-489, by the homology alignment algorithm of Needleman and Wunsch (1970) I. Mol. Nat'l. Acad. Sci. USA 85:2444-2448, by computerized implementations of these algorithms visual inspection (see, e.g., Ausubel, et al. (eds. 1995 and supplements) Current Protocols in number of contiguous positions selected from the group consisting typically of from 20 to Biol. 48:443-453, by the search for similarity method of Pearson and Lipman (1988) Proc. Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and e.g., by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Molecular Biology Lippincott.

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described in Altschul, et al. (1977) Nuc. Acids Res. 25:3389-3402 and Altschul, et al. (1990) Preferred examples of algorithms that are suitable for determining percent sequence identity and sequence similarity include the BLAST and BLAST 2.0 algorithms, which are

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1. Mol. Biol. 215:403-410. BLAST and BLAST 2.0 are used, with the parameters described herein, to determine percent sequence identity for the nucleic acids and proteins of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This

- words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short referred to as the neighborhood word score threshold (Altschul, et al., supra). These initial cumulative alignment score can be increased. Cumulative scores are calculated using, e.g., neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the for nucleotide sequences, the parameters M (reward score for a pair of matching residues; 2
  - sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word lue to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the quantity X from its maximum achieved value; the cumulative score goes to zero or below, sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) lways > 0) and N (penalty score for mismatching residues; always < 0). For amino acid hits in each direction are halted when: the cumulative alignment score falls off by the 13
- (see Henikoff and Henikoff (1989) Proc. Nat'l Acad. Sci. USA 89:10915-919) alignments (B) defaults a word length of 3, and expectation (B) of 10, and the BLOSUM62 scoring matrix comparison of both strands. For amino acid sequences, the BLASTP program uses as uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=4 and a of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

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probability (P(N)), which provides an indication of the probability by which a match between wo nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin and Altschul (1993) Proc. Nat'l Acad. Sci. USA 90:5873of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably ess than about 0.01, and most preferably less than about 0.001. Log values may be large 5887). One measure of similarity provided by the BLAST algorithm is the smallest sum negative numbers, e.g., 5, 10, 20, 30, 40, 40, 70, 90, 110, 150, 170, etc. 2 23

An indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, e.g., where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules or their complements hybridize to each other under stringent conditions, as described below. Yet another indication that two nucleic acid sequences are substantially identical is that the same primers can be used to amplify the sequences.

A "host cell" is a naturally occurring cell or a transformed cell that contains an expression vector and supports the replication or expression of the expression vector. Host cells may be cultured cells, explants, cells in vivo, and the like. Host cells may be prokaryotic cells such as E. coli, or eukaryotic cells such as yeast, insect, amphibian, or mammalian cells, such as CHO, HeLa, and the like (see, e.g., the American Type Culture Collection catalog or web site, www.atcc.org).

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The terms "isolated," "purified," or "biologically pure" refer to material that is substantially or essentially free from components that normally accompany it as found in its native state. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein or nucleic acid that is the predominant species present in a preparation is substantially purified. In particular, an isolated nucleic acid is separated from some open reading frames that naturally flank the gene and encode proteins other than protein encoded by the gene. The term "purified" in some embodiments denotes that a nucleic acid or protein is at least 85% pure, more preferably at least 95% pure, and most preferably at least 99% pure. "Purify" or "purification" in other embodiments means removing at least one contaminant from the composition to be purified. In this sense, purification does not require that the purified compound be homogenous, e.g., 100% pure.

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The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers, those containing modified residues, and non-naturally occurring amino acid polymers.

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WO 02/102235

PCT/US02/19297

The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function similarly to the naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline,  $\gamma$ -

5 carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, e.g., an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs may have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions similarly to a naturally occurring amino acid.

Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical

Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

"Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical or associated, e.g., naturally contiguous, sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode most proteins. For instance, the codons GCA, GCC, GCG, and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can

be altered to another of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes silent variations of the nucleic acid. In certain contexts each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and

30 TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, a silent variation of a nucleic acid which encodes a polypeptide is implicit in a described sequence with respect to the expression product, but not necessarily with respect to actual probe sequences.

substitution of an amino acid with a chemically similar amino acid. Conservative substitution conservatively modified variants are in addition to and do not exclude polymorphic variants, As to amino acid sequences, one of skill will recognize that individual substitutions, encoded sequence is a "conservatively modified variant" where the alteration results in the interspecies homologs, and alleles of the invention. Typically conservative substitutions for deletions, or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds, or deletes a single amino acid or a small percentage of amino acids in the one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) tables providing functionally similar amino acids are well known in the art. Such

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Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, e.g., Creighton (1984) Proteins Freeman). 2

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Macromolecular structures such as polypeptide structures can be described in terms of sections of lesser organization such as stretches of eta-sheet and lpha-helices. "Tertiary structure" Domains are portions of a polypeptide that often form a compact unit of the polypeptide and association of independent tertiary units. Anisotropic terms are also known as energy terms. Alberts, et al. (2001) Molecular Biology of the Cell (4th ed.) Garland Pub.; and Cantor and refers to the complete three dimensional structure of a polypeptide monomer. "Quaternary are typically 25 to approximately 500 amino acids long. Typical domains are made up of structure" refers to the three dimensional structure formed, usually by the non-covalent Macromolecules Freeman. "Primary structure" refers to the amino acid sequence of a various levels of organization. For a general discussion of this organization, see, e.g., particular peptide. "Secondary structure" refers to locally ordered, three dimensional structures within a polypeptide. These structures are commonly known as domains. Schimmel (1980) Biophysical Chemistry Part I: The Conformation of Biological

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typically from about 5, 6, 7, 8, 9, 10, 12, 15, 25, 30, 40, 50, or more nucleotides in length, up used herein means at least two nucleotides covalently linked together. Oligonucleotides are to about 100 nucleotides in length. Nucleic acids and polynucleotides are a polymers of any "Nucleic acid" or "oligonucleotide" or "polynucleotide" or grammatical equivalents length, including longer lengths, e.g., 200, 300, 500, 1000, 2000, 3000, 5000, 7000, 10,000, etc. A nucleic acid of the present invention will generally contain phosphodiester bonds,

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although in some cases, nucleic acid analogs are included that may have at least one different

methylphosphoroamidite linkages (see Eckstein (1992) <u>Oligonucleotides and Analogues: A</u> Practical Approach Oxford University Press); and peptide nucleic acid backbones and inkage, e.g., phosphoramidate, phosphorothioate, phosphorodithioate, or O-

- icids containing one or more carbocyclic sugars are also included within one definition of Carbohydrate Modifications in Antisense Research ASC Symposium Series 580. Nucleic linkages. Other analog nucleic acids include those with positive backbones; non-ionic backbones, and non-ribose backbones, including those described in U.S. Patent Nos. 5,235,033 and 5,034,506, and Chapters 6 and 7 of Sanghvi and Cook (eds. 1994) 2
- nucleic acids. Modifications of the ribose-phosphate backbone may be done for a variety of analogs can be made; alternatively, mixtures of different nucleic acid analogs, and mixtures environments or as probes on a biochip. Mixtures of naturally occurring nucleic acids and easons, e.g., to increase the stability and half-life of such molecules in physiological of naturally occurring nucleic acids and analogs may be made.
- etsinger (1970) J. Org. Chem. 35:3800-3803; Sprinzl, et al. (1977) Eur. J. Bjochem. 81:579phosphoramidate (Beaucage, et al. (1993) Tetrahedron 49:1925-1963 and references therein; 589; Letsinger, et al. (1986) Nucl. Acids Res. 14:3487-499; Sawai, et al. (1984) Chem. Lett. 805, Letsinger, et al. (1988) I. Am. Chem. Soc. 110:4470-4471; and Pauwels, et al. (1986), A variety of references disclose such nucleic acid analogs, including, e.g., 15
  - 19:1437-441; and U.S. Patent No. 5,644,048), phosphorodithioate (Brill, et al. (1989) L.Am. nucleic acid backbones and linkages (see Egholm (1992) J. Am. Chem. Soc. 114:1895-897; Oligonucleotides and Analogues: A Practical Approach Oxford Univ. Press), and peptide Chem. Soc. 111:2321-2322), O-methylphophoroamidite linkages (see Eckstein (1992) Chemica Scripta 26:141-149), phosphorothioate (Mag, et al. (1991) Nucl. Acids Res. 2
- ,386,023, 5,637,684, 5,602,240, 5,216,141 and 4,469,863; Kiedrowshi, et al. (1991) Angew. eference). Other analog nucleic acids include those with positive backbones (Denpcy, et al. Meier, et al. (1992) Angew. Chem. Int. Ed. Engl. 31:1008-1010; Nielsen (1993) Nature, (1995) Proc. Nat'l Acad. Sci. USA 92:6097-101; non-ionic backbones (U.S. Patent Nos. 365:566-568; Carlsson, et al. (1996) Nature 380:207, each of which is incorporated by 23
- Series 580; Mesmaeker, et al. (1994) Bioorganic and Medicinal Chem. Lett. 4:395-398; Jeffs, 1471; Jung, et al. (1994) Nucleoside and Nucleotide 13:1597; Chapters 2 and 3, in Sanghvi and Cook (eds. 1994) Carbohydrate Modifications in Antisense Research ASC Symposium Chem. Intl. Bd. English 30:423-426; Letsinger, et al. (1988) J. Am. Chem. Soc. 110:4470-30

WO 02/10223

PCT/US02/19297

xxx) and non-ribose backbones, including those described in U.S. Patent Nos. 5,235,033 and Modifications in Antisense Research ASC Symposium Series 580. Nucleic acids containing et al. (1994) <u>J. Biomolecular NMR</u> 34:17-xx; Horn, et al. (1996) <u>Tetrahedron Lett.</u> 37:743one or more carbocyclic sugars are also included within one definition of nucleic acids (see described in Rawls (p. 35 June 2, 1997) C&E News. Each of these references is hereby Jenkins, et al. (1995) Chem. Soc. Rev. pp 169-176). Several nucleic acid analogs are 5,034,506, and Chapters 6 and 7, in Sanghvi and Cook (eds. 1994) Carbohydrate expressly incorporated by reference.

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Particularly preferred are peptide nucleic acids (PNA) which includes peptide nucleic backbones is relatively insensitive to salt concentration. In addition, PNAs are not degraded contrast to the highly charged phosphodiester backbone of naturally occurring nucleic acids. kinetics. PNAs have larger changes in the melting temperature  $(T_{f m})$  for mismatched versus perfectly matched base pairs. DNA and RNA typically exhibit a 2-4° C drop in  $T_{
m m}$  for an This results in two advantages. First, the PNA backbone exhibits improved hybridization acid analogs. These backbones are substantially non-ionic under neutral conditions, in Similarly, due to their non-ionic nature, hybridization of the bases attached to these internal mismatch. With the non-ionic PNA backbone, the drop is closer to 7-9° C. by cellular enzymes, and thus can be more stable.

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portions of both double stranded or single stranded sequence. As will be appreciated by those nucleic acid may contain combinations of deoxyribo- and ribo-nucleotides, and combinations The nucleic acids may be single stranded or double stranded, as specified, or contain in the art, the depiction of a single strand also defines the sequence of the complementary strand; thus the sequences described herein also provide the complement of the sequence. naturally occurring analog structures. Thus, e.g., the individual units of a peptide nucleic nucleosides such as amino modified nucleosides. In addition, "nucleoside" includes non-"nucleoside" includes nucleotides and nucleoside and nucleotide analogs, and modified The nucleic acid may be DNA, both genomic and cDNA, RNA, or a hybrid, where the hypoxanthine, isocytosine, isoguanine, etc. "Transcript" typically refers to a naturally occurring RNA, e.g., a pre-mRNA, hnRNA, or mRNA. As used herein, the term of bases, including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine acid, each containing a base, are referred to herein as a nucleoside. 30

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A "label" or a "detectable moiety" is a composition detectable by spectroscopic,

as commonly used in an ELISA), biotin, digoxigenin, or haptens and proteins or other entities the ovarian cancer nucleic acids, proteins and antibodies at any position. Any method known in the art for conjugating the antibody to the label may be employed, including those methods example, useful labels include 32P, fluorescent dyes, electron-dense reagents, enzymes (e.g., detect antibodies specifically reactive with the peptide. The labels may be incorporated into which can be made detectable, e.g., by incorporating a radiolabel into the peptide or used to described by Hunter, et al. (1962) Nature 144:945-xxx; David, et al. (1974) Biochemistry 13:1014-1021; Pain, et al. (1981) <u>J. Immunol. Meth.</u> 40:219-230; and Nygren (1982) <u>J.</u> photochemical, biochemical, immunochemical, chemical, or other physical means. For Ś

including radioactive compounds, fluorescent compounds, an enzyme or substrate, tags such bound (or linked, or conjugated), either covalently, through a linker or a chemical bond, or antibody. The "effector" can be a variety of molecules including, e.g., detection moieties An "effector" or "effector moiety" or "effector component" is a molecule that is non-covalently, through ionic, van der Waals, electrostatic, or hydrogen bonds, to an as epitope tags, a toxin; activatable moieties, a chemotherapeutic agent; a lipase; an antibiotic; or a radioisotope emitting "hard" e.g., beta radiation. 15

Histochem, and Cytochem, 30:407-412.

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Waals, electrostatic, or hydrogen bonds to a label such that the presence of the probe may be using high affinity interactions may achieve the same results where one of a pair of binding covalently, through a linker or a chemical bond, or non-covalently, through ionic, van der detected by detecting the presence of the label bound to the probe. Alternatively, method A "labeled nucleic acid probe or oligonucleotide" is one that is bound, either partners binds to the other, e.g., biotin, streptavidin.

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chemical bonds, usually through complementary base pairing, usually through hydrogen bond aybridization. Thus, e.g., probes may be peptide nucleic acids in which the constituent bases bases (7-deazaguanosine, inosine, etc.). In addition, the bases in a probe may be joined by a As used herein a "nucleic acid probe or oligonucleotide" is a nucleic acid capable of inkage other than a phosphodiester bond, so long as it does not functionally interfere with sequences lacking complete complementarity with the probe sequence depending upon the binding to a target nucleic acid of complementary sequence through one or more types of formation. As used herein, a probe may include natural (e.g., A, G, C, or T) or modified are joined by peptide bonds rather than phosphodiester linkages. Probes may bind target 52 8

PCT/US02/19297

WO 02/102235

PCT/US02/19297

stringency of the hybridization conditions. The probes are preferably directly labeled, e.g., with isotopes, chromophores, lumiphores, chromogens, or indirectly labeled such as with biotin to which a streptavidin complex may later bind. By assaying for the presence or absence of the probe, one can detect the presence or absence of the select sequence or subsequence. Diagnosis or prognosis may be based at the genomic level, or at the level of RNA or protein expression.

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recombinant for the purposes of the invention. Similarly, a "recombinant protein" is a protein protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modiffed by organism, it will replicate non-recombinantly, e.g., using the in vivo cellular machinery of the operably linkage of different sequences is achieved. Thus an isolated nucleic acid, in a linear cells express genes that are not found within the native (non-recombinant) form of the cell or understood that once a recombinant nucleic acid is made and reintroduced into a host cell or the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, e.g., recombinant made using recombinant techniques, e.g., through the expression of a recombinant nucleic normally joined, are both considered recombinant for the purposes of this invention. It is The term "recombinant" when used with reference, e.g., to a cell, or nucleic acid, polymerases and endonucleases, in a form not normally found in nature. In this manner, recombinantly, although subsequently replicated non-recombinantly, are still considered host cell rather than in vitro manipulations; however, such nucleic acids, once produced expressed at all. By the term "recombinant nucleic acid" herein is meant nucleic acid, form, or an expression vector formed in vitro by ligating DNA molecules that are not express native genes that are otherwise abnormally expressed, under expressed or not originally formed in vitro, in general, by the manipulation of nucleic acid, e.g., using acid as depicted above.

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The term: 'heterologous' when used with reference to portions of a nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not normally found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences, e.g., from unrelated genes arranged to make a new functional nucleic acid, e.g., a promoter from one source and a coding region from another source. Similarly, a heterologous protein will often refer to two or more subsequences that are not found in the same relationship to each other in nature (e.g., a fusion protein).

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A "promoter" is defined as an array of nucleic acid control sequences that direct transcription of a nucleic acid. As used herein, a promoter includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor

5 elements, which can be located as much as several thousand base pairs from the start site of transcription. A "constitutive" promoter is a promoter that is active under most environmental and developmental conditions. An "inducible" promoter is a promoter that is active under environmental or developmental regulation. The term "operably linked" refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter, or array of transcription factor binding sites) and a second nucleic acid sequence, e.g., wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

An "expression vector" is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a host cell. The expression vector can be part of a plasmid, virus, or nucleic acid fragment. Typically, the expression vector includes a nucleic acid to be transcribed operably linked to a promoter.

The phrase "selectively (or specifically) hybridizes to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent hybridization conditions when that sequence is present in a complex mixture (e.g., total cellular or library DNA or RNA).

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The plurase "stringent hybridization conditions" refers to conditions under which a probe will hybridize to its target subsequence, typically in a complex mixture of nucleic acids, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in "Overview of principles of hybridization and the strategy of nucleic acid assays" in Tijssen (1993)

Hybridization with Nucleic Probes (Laboratory Techniques in Biochemistry and Molecular Biology) (vol. 24) Elsevier. Generally, stringent conditions are selected to be about 5-10° C

30 lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target

WO 02/102235

PCT/US02/19297

sequence at equilibrium (as the target sequences are present in excess, at  $T_{\rm m}$ , 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt

concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30° C for

s short probes (e.g., 10 to 50 nucleotides) and at least about 60° C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of

destabilizing agents such as formamide. For selective or specific hybridization, a positive

signal is typically at least two times background, preferably 10 times background hybridization. Exemplary stringent hybridization conditions can be as following: 50%

10 formamide, 5x SSC, and 1% SDS, incubating at 42° C, or, 5x SSC, 1% SDS, incubating at 65° C, with wash in 0.2x SSC, and 0.1% SDS at 65° C. For PCR, a temperature of about 36°

65° C, with wash in 0.2x SSC, and 0.1% SDS at 65° C. For PCR, a temperature of about 36° C is typical for low stringency amplification, although annealing temperatures may vary.

between about 32-48° C depending on primer length. For high stringency PCR amplification, a temperature of about 62° C is typical, although high stringency annealing temperatures can

a temperature of about 62° C is typical, although high stringency annealing temperatures can 15 range from about 50° C to about 65° C, depending on the primer length and specificity.

Typical cycle conditions for both high and low stringency amplifications include a denaturation phase of 90-95° C for 30-120 sec, an annealing phase lasting 30-120 sec, and an

extension phase of about 72° C for 1-2 min. Protocols and guidelines for low and high

stringency amplification reactions are available, e.g., in Innis, et al. (1990) <u>PCR Protocols: A</u>

20 Guide to Methods and Applications Academic Press, N.Y.

Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, e.g., when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. In such cases, the nucleic acids typically hybridize under moderately stringent hybridization conditions. Exemplary "moderately stringent hybridization conditions" include a hybridization in a buffer of 40% formamide, 1 M NaCl, 1% SDS at 37° C, and a wash in 1X SSC at 45° C. A positive hybridization is at least twice background. Alternative hybridization and wash conditions can be utilized to provide conditions of similar stringency. Additional guidelines for determining hybridization parameters are provided, e.g., Ausubel, et al. (ed. 1991 and supplements) Current Protocols in Molecular Biology Lippincott.

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The phrase "functional effects" in the context of assays for testing compounds that

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modulate activity of an ovarian cancer protein includes the determination of a parameter that is indirectly or directly under the influence of the ovarian cancer protein or nucleic acid, e.g., a functional, physical, physiological, or chemical effect, such as the ability to decrease ovarian cancer. It includes ligand binding activity; cell growth on soft agar; anchorage dependence; contact inhibition and density limitation of growth; cellular proliferation; cellular transformation; growth factor or serum dependence; tumor specific marker levels; invasiveness into Matrigel; tumor growth and metastasis in vivo; mRNA and protein expression in cells undergoing metastasis, and other characteristics of ovarian cancer cells. "Functional effects" include in vitro, in vivo, and ex vivo activities.

10 By "determining the functional effect" is meant assaying for a compound that increases or decreases a parameter that is indirectly or directly under the influence of an ovarian cancer protein sequence, e.g., functional, enzymatic, physical, physiological, and chemical effects. Such functional effects can be measured by any means known to those skilled in the art, e.g., changes in spectroscopic characteristics (e.g., fluorescence,

absorbance, refractive index), hydrodynamic (e.g., shape), chromatographic, or solubility properties for the protein, measuring inducible markers or transcriptional activation of the ovarian cancer protein; measuring binding activity or binding assays, e.g., binding to antibodies or other ligands, and measuring cellular proliferation. Determination of the functional effect of a compound on ovarian cancer can also be performed using ovarian

20 cancer assays known to those of skill in the art such as an in vitro assays, e.g., cell growth on soft agar, anchorage dependence; contact inhibition and density limitation of growth; cellular proliferation; cellular transformation; growth factor or serum dependence; tumor specific marker levels; invasiveness into Matrigel; tumor growth and metastasis in vivo; mRNA and protein expression in cells undergoing metastasis, and other characteristics of ovarian cancer

cells. The functional effects can be evaluated by means known to those skilled in the art, e.g., microscopy for quantitative or qualitative measures of alterations in morphological features, measurement of changes in RNA or protein levels for ovarian cancer-associated sequences, measurement of RNA stability, or identification of downstream or reporter gene expression (CAT, luciferase, β-gal, GFP, and the like), e.g., via chemiluminescence, fluorescence,

colonimetric reactions, antibody binding, inducible markers, and ligand binding assays.

"Inhibitors", "activators", and "modulators" of ovarian cancer polynucleotide and polypeptide sequences are used to refer to activating, inhibitory, or modulating molecules or compounds identified using in vitro and in vivo assays of ovarian cancer polynucleotide and

polypeptide sequences. Inhibitors are compounds that, e.g., bind to, partially or totally block activity, decrease, prevent, delay activation, inactivate, desensitize, or down regulate the activity or expression of ovarian cancer proteins, e.g., antagonists. Antisense or inhibitory nucleic acids may inhibit expression and subsequent function of the protein. "Activators" are compounds that increase, open, activate, facilitate, enhance activation, sensitize, agonize, or up regulate ovarian cancer protein activity. Inhibitors, activators, or modulators also include genetically modified versions of ovarian cancer proteins, e.g., versions with altered activity, as well as naturally occurring and synthetic ligands, antagonists, agonists, antibodies, small chemical molecules, and the like. Assays for inhibitors and activators include, e.g.,

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expressing the ovarian cancer protein in vitro, in cells, or cell membranes, applying putative modulator compounds, and then determining the functional effects on activity, as described above. Activators and inhibitors of ovarian cancer can also be identified by incubating ovarian cancer cells with the test compound and determining increases or decreases in the expression of one or more ovarian cancer proteins, e.g., 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50, or more ovarian cancer proteins, such as ovarian cancer proteins encoded by the

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sequences set out in Tables 1-26.

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Samples or assays comprising ovarian cancer proteins that are treated with a potential activator, inhibitor, or modulator are compared to control samples without the inhibitor, activator, or modulator to examine the extent of inhibition. Control samples (untreated with inhibitors) are assigned a relative protein activity value of 100%. Inhibition of a polypeptide is achieved when the activity value relative to the control is about 80%, preferably 50%, more preferably 25% or less. Activation of an ovarian cancer polypeptide is achieved when the activity value relative to the control (untreated with activators) is 110%, more preferably 150%, more preferably 1000-3000% higher.

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The phrase "changes in cell growth" refers to a change in cell growth and proliferation characteristics in vitro or in vivo, e.g., cell viability, formation of foci, anchorage independence, semi-solid or soft agar growth, change in contact inhibition or density limitation of growth, loss of growth factor or serum requirements, change in cell morphology, gain or loss of immortalization, gain or loss of tumor abecific markers, ability to form or suppress tumors when injected into suitable animal hosts, and/or immortalization of the cell. See, e.g., pp. 231-241 in Freshney (1994) Culture of Animal Cells: A Manual of Basic Technique (3d ed.) Wiley-Liss.

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"Tumor cell" refers to pre-cancerous, cancerous, and normal cells in a tumor.

"Cancer cells," "transformed" cells or "transformation" in tissue culture, refers to spontaneous or induced phenotypic changes that do not necessarily involve the uptake of new genetic material. Although transformation can arise from infection with a transforming virus and incorporation of new genomic DNA, or uptake of exogenous DNA, it can also arise spontaneously or following exposure to a carcinogen, thereby mutating an endogenous gene. Transformation is typically associated with phenotypic changes, such as immortalization of cells, aberrant growth control, non-morphological changes, and/or malignancy. See,

Freshney (1994) Culture of Animal Cells.

immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen.

The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD, and IgE, respectively. Typically, the antigen-binding region of an antibody or its functional equivalent will be most critical in specificity and affinity of binding. See, e.g., Paul (ed. 1999) Fundamental Immunology (4th ed.) Raven.

An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (VL) and variable heavy chain (VH) refer to these light and heavy chains respectively.

fragments produced by digestion with various peptidases. Thus, e.g., pepsin digests an antibody below the disulfide linkages in the hinge region to produce F(ab)'2, a dimer of Fab which itself is a light chain joined to VH-CH1 by a disulfide bond. The F(ab)'2 may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the F(ab)'2 dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region. See Paul (ed. 1999) Fundamental Immunology (4th ed.) Raven. While various antibody fragments are defined in terms of the digestion of an intact antibody,

one of skill will appreciate that such fragments may be synthesized de novo either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized de novo using recombinant DNA methodologies (e.g., single chain Fv) or those identified using phage display libraries. See, e.g., McCafferty, et al. (1990) Nature 148.552.554

For preparation of antibodies, e.g., recombinant, monoclonal, or polyclonal antibodies, many techniques known in the art can be used (see, e.g., Kohler and Milstein (1975) Nature 256:495-497; Kozbor, et al. (1983) Immunology Today 4:72; Cole, et al., pp. 77-96 in Reisfeld and Sell (1985) Monoclonal Antibodies and Cancer Therapy Liss; Coligan (1991) Current Protocols in Immunology Lippincott; Harlow and Lane (1988) Antibodies: A Laboratory Manual CSH Press, and Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed.) Academic Press. Techniques for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce antibodies to polypeptides of this invention. Transgenic mice, or other organisms, e.g., other mammals, may be used to express humanized antibodies. Alternatively, phage display technology can be used to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens. See, e.g., McCafferty, et al. (1990) Nature 348:552-554; and Marks, et al. (1992) Biotechnology 10:779-783.

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20 A "chimeric antibody" is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity.

# Identification of ovarian cancer-associated sequences

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In one aspect, the expression levels of genes are determined in different patient samples for which diagnosis information is desired, to provide expression profiles. An expression profile of a particular sample is essentially a "fingerprint" of the state of the sample; while two states may have any particular gene similarly expressed, the evaluation of a number of genes simultaneously allows the generation of a gene expression profile that is 26

WO 02/102235

PCT/US02/19297

characteristic of the state of the cell. That is, normal tissue (e.g., normal ovarian or other tissue) may be distinguished from cancerous or metastatic cancerous tissue of the ovarian, or ovarian cancer tissue or metastatic ovarian cancerous tissue can be compared with tissue samples of ovarian and other tissues from surviving cancer patients. By comparing expression profiles of tissue in known different ovarian cancer states, information regarding which genes are important (including both up- and down-regulation of genes) in each of these states is obtained. Molecular profiling may distinguish subtypes of a currently collective disease designation, e.g., different forms of a cancer.

The identification of sequences that are differentially expressed in ovarian cancer versus non-ovarian cancer tissue allows the use of this information in a number of ways. For example, a particular treatment regime may be evaluated: does a chemotherapeutic drug act to down-regulate ovarian cancer, and thus tumor growth or recurrence, in a particular patient. Alternatively, does existing treatment induce expression of a target. Similarly, diagnosis and treatment outcomes may be done or confirmed by comparing patient samples with the known expression profiles. Metastatic tissue can also be analyzed to dotcrmine the stage of ovarian cancer in the tissue or origin of the primary tumor. Furthermore, these gene expression profiles (or individual genes) allow screening of drug candidates with an eye to mimicking or altering a particular expression profile; e.g., screening can be done for drugs that suppress the ovarian cancer expression profile. This may be done by making biochips comprising sets of

ovarian cancer expression profile. This may be done by making biochips comprising sets of
the important ovarian cancer genes, which can then be used in these screens. These methods
can also be based on evaluating protein expression; that is, protein expression levels of the
ovarian cancer proteins can be evaluated for diagnostic purposes or to screen candidate
agents. In addition, the ovarian cancer nucleic acid sequences can be administered for gene
therapy purposes, including the administration of antisense or RNAi nucleic acids, or the
ovarian cancer proteins (including antibodies and other modulators thereof) administered as
therapeutic drugs.

Thus the present invention provides nucleic acid and protein sequences that are differentially expressed in ovarian cancer relative to normal tissues and/or non-malignant tissues, herein termed "ovarian cancer sequences." As outlined below, ovarian cancer sequences include those that are up-regulated (e.g., expressed at a higher level) in ovarian cancer, as well as those that are down-regulated (e.g., expressed at a lower level). In a preferred embodiment, the ovarian cancer sequences are from humans; however, as will be appreciated by those in the art, ovarian cancer sequences from other organisms may be useful

in animal models of disease and drug evaluation; thus, other ovarian cancer sequences are provided, from vertebrates, including mammals, including rodents (rats, mice, hamsters, guinea pigs, etc.), primates, farm animals (including sheep, goats, pigs, cows, horses, etc.) and pets (e.g., dogs, cats, etc.). Ovarian cancer sequences, e.g., counterpart genes, from other organisms may be obtained using the techniques outlined below.

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Ovarian cancer sequences can include both nucleic acid and amino acid sequences.

Ovarian cancer nucleic acid sequences are useful in a variety of applications, including diagnostic applications, which will detect naturally occurring nucleic acids. Screening applications; e.g., biochips comprising nucleic acid probes or PCR microtiter plates with selected probes to the ovarian, cancer sequences, are also provided.

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An ovarian cancer sequence can be initially identified by substantial nucleic acid and/or amino acid sequence homology to the ovarian cancer sequences outlined herein. Such homology can be based upon the overall nucleic acid or amino acid sequence, and is generally determined as outlined below, using either homology programs or hybridization conditions.

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For identifying ovarian cancer-associated sequences, the ovarian cancer screen typically includes comparing genes identified in different tissues, e.g., normal and cancerous tissues, or tumor tissue samples from patients who have metastatic disease vs. non metastatic tissue. Other suitable tissue comparisons include comparing ovarian cancer samples with metastatic cancer samples from other cancers, such as lung, ovarian, gastrointestinal cancers, etc. Samples of different stages of ovarian cancer, e.g., survivor tissue, drug resistant states, and tissue undergoing metastasis, are applied to biochips comprising nucleic acid probes. The samples are first microdissected, if applicable, and treated for the preparation of mRNA. Suitable biochips are commercially available, e.g., from Affymetrix. Gene expression profiles as described herein are generated and the data analyzed.

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In one embodiment, the genes showing changes in expression as between normal and disease states are compared to genes expressed in other normal tissues, preferably normal ovarian, but also including, and not limited to, lung, heart, brain, liver, ovarian, kidney, muscle, colon, small intestine, large intestine, spleen, bone, and/or placenta. In a preferred embodiment, those genes identified during the ovarian cancer screen that are expressed in any significant amount in other tissues are removed from the profile, although in some embodiments, expression in non-essential tissues may be tolerated. That is, when screening for drugs, it is usually preferable that the target be disease specific, to minimize possible side

WO 02/102235

PCT/US02/19297

effects by interaction with target present in other organs.

In a preferred embodiment, ovarian cancer sequences are those that are up-regulated in ovarian cancer; that is, the expression of these genes is higher in the ovarian cancer tissue as compared to non-cancerous tissue. "Up-regulation" as used herein often means at least about a two-fold change, preferably at least about a three fold change, with at least about five-fold or higher being preferred. Other embodiments are directed to sequences up regulated in non-malignant conditions relative to normal.

Unigene cluster identification numbers and accession numbers herein refer to the GenBank sequence database and the sequences of the accession numbers are hereby expressly incorporated by reference. GenBank is known in the art, see, e.g., Benson, et al. (1998) Nucl. Acids Res. 26:1-7; and http://www.ncbi.nlm.nih.gov/. Sequences are also available in other databases, e.g., Buropean Molecular Biology Laboratory (EMBL) and DNA Database of Japan (DDBJ). In some situations, the sequences may be derived from assembly of available sequences or be predicted from genomic DNA using exon prediction algorithms, e.g., FGENESH. See Salamov and Solovyev (2000) Genome Res. 10:516-522. In other situations, sequences have been derived from cloning and sequencing of isolated

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In another preferred embodiment, ovarian cancer sequences are those that are downregulated in ovarian cancer, that is, the expression of these genes is lower in ovarian cancer tissue as compared to non-cancerous tissue. "Down-regulation" as used herein often means at least about a two-fold change, preferably at least about a three-fold change, with at least about five-fold or higher being preferred.

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#### Informatics

25 The ability to identify genes that are over or under expressed in ovarian cancer can additionally provide high-resolution, high-sensitivity datasets which can be used in the areas of diagnostics, therapeutics, drug development, pharmacogenetics, protein structure, biosensor development, and other related areas. Expression profiles can be used in diagnostic or prognostic evaluation of patients with ovarian cancer. Subcellular toxicological information can be generated to better direct drug structure and activity correlation (see

information can be generated to better direct drug structure and activity correlation (see Anderson (June 11-12, 1998) Pharmaceutical Proteomics: Targets, Mechanism, and Function, paper presented at the IBC Proteomics conference, Coronado, CA) or in a biological sensor device to predict the likely toxicological effect of chemical exposures and likely tolerable

WO 02/102235

PCT/US02/19297

datasets relevant to other biomolecules and bioactive agents (e.g., nucleic acids, saccharides, exposure thresholds (see U.S. Patent No. 5,811,231). Similar advantages accrue from lipids, drugs, and the like).

at least one set of assay data. The data contained in the database is acquired, e.g., using array such as a personal computer, but is preferably distributed on a wide area network, such as the analysis either singly or in a library format. The database can be in a form in which data can Thus, in another embodiment, the present invention provides a database that includes maintained on any electronic device allowing for the storage of and access to the database, be maintained and transmitted, but is preferably an electronic database, and can be World Wide Web.

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The focus of the present section on databases that include peptide sequence data is for clarity of illustration only. It will be apparent to those of skill in the art that similar databases can be assembled for any assay data acquired using an assay of the invention.

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The compositions and methods for identifying and/or quantitating the relative and/or absolute abundance of a variety of molecular and macromolecular species from a biological with pathological conditions, predisposition to disease, drug testing, therapeutic monitoring, sequences described herein, provide an abundance of information which can be correlated status, and outcome data, among others. Although data generated from the assays of the gene-disease causal linkages, identification of correlates of immunity and physiological sample undergoing ovarian cancer, e.g., the identification of ovarian cancer-associated invention is suited for manual review and analysis, in a preferred embodiment, data processing using high-speed computers is utilized.

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sequence similar to a sequence data item in a gene database based on the degree of similarity in the art. For example, U.S. Patents 6,023,659 and 5,966,712 disclose a relational database An array of methods for indexing and retrieving biomolecular information is known system for storing biomolecular sequence information in a manner that allows sequences to obtaining full-length sequences from the collection of partial length sequences. U.S. Patent catalogued and searched according to association with one or more sequencing projects for between a key sequence and a target sequence. U.S. Patent 5,538,897 discloses a method be catalogued and searched according to one or more protein function hierarchies. U.S. information in a format that allows a collection of partial-length DNA sequences to be Patent 5,953,727 discloses a relational database having sequence records containing 5,706,498 discloses a gene database retrieval system for making a retrieval of a gene 22 2

using mass spectroscopy fragmentation patterns of peptides to identify amino acid sequences in computer databases by comparison of predicted mass spectra with experimentally-derived mass spectra using a closeness-of-fit measure. U.S. Patent 5,926,818 discloses a multidimensional database comprising a functionality for multi-dimensional data analysis

projected and actual data according to more than one consolidation path or dimension. U.S. fields stored in a hierarchical topological map which can be viewed as a tree structure or as record are divided into two classes, navigational and informational data, with navigational Patent 5,295,261 reports a hybrid database structure in which the fields of each database described as on-line analytical processing (OLAP), which entails the consolidation of the merger of two or more such tree structures. S

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et al. (eds 1997) Introduction to Computational Molecular Biology Brooks/Cole; Misener and Guide to the Analysis of Genes and Proteins (2d ed.) Wiley-Liss; Rashidi and Buehler (1999) Bioinformatics: Basic Applications in Biological Science and Medicine CRC Press; Setubal, Bioinformatics: Sequence and Genome Analysis CSH Press, NY; Durbin, et al. (eds. 1999) Cambridge Univ. Press; Baxevanis and Ocullette (eds. 1998) Bioinformatics: A Practical Krawetz (eds. 2000) Bioinformatics: Methods and Protocols Humana Press; Higgins and Taylor (eds. 2000) Bioinformatics: Sequence, Structure, and Databanks; A Practical Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids Fundamentals of bioinformatics are provided, e.g., in Mount, et al. (2001)

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Biocomputing and the Internet Baton Pub.; Han and Kamber (2000) Data Mining: Concepts and Techniques Kaufmann Pub.; and Waterman (1995) Introduction to Computational Approach Oxford Univ. Press; Brown (2001) Bioinformatics: A Biologist's Guide to Biology: Maps, Sequences, and Genomes Chap and Hall. 2

with data specifying the source of the target-containing sample from which each sequence software for storing in computer-retrievable form assay data records cross-tabulated, e.g., The present invention provides a computer database comprising a computer and specificity record was obtained. 25

another tissue specimen to be analyzed for ovarian cancer. In another variation, assay records cross-tabulate one or more of the following parameters for a target species in a sample: (1) a is from a control tissue sample known to be free of pathological disorders. In a variation, at least one of the sources is a known pathological tissue specimen, e.g., a neoplastic lesion or In an exemplary embodiment, at least one of the sources of target-containing sample unique identification code, which can include, e.g., a target molecular structure and/or

WO 02/102235

PCT/US02/19297

characteristic separation coordinate (e.g., electrophoretic or genomic position coordinates); (2) sample source; and (3) absolute and/or relative quantity of target species present in the sample.

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and a charge storage area, which may be on the transistor). In one embodiment, the invention data storage arrays. Typically, the target data records are stored as a bit pattern in an array of magnetic domains on a magnetizable medium or as an array of charge states or transistor gate magneto-optical disks, DRAM, SRAM, SGRAM, SDRAM, RDRAM, DDR RAM, magnetic bubble memory devices, and other data storage devices, including CPU registers and on-CPU provides such storage devices, and computer systems built therewith, comprising a bit pattern encoding a protein expression fingerprint record comprising unique identifiers for at least 10 The invention also provides for the storage and retrieval of a collection of target data states, such as an array of cells in a DRAM device (e.g., each cell comprised of a transistor in a computer data storage apparatus, which can include magnetic disks, optical disks, target data records cross-tabulated with target source.

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or retrieved from a computer storage device or database and at least one other sequence. The computerized comparison between a peptide or nucleic acid sequence assay record stored in comparison can include a sequence analysis or comparison algorithm or computer program embodiment thereof (e.g., FASTA, TFASTA, GAP, BESTFIT) and/or the comparison may. method for identifying related peptide or nucleic acid sequences, comprising performing a When the target is a peptide or nucleic acid, the invention preferably provides a be of the relative amount of a peptide or nucleic acid sequence in a pool of sequences determined from a polypeptide or nucleic acid sample of a specimen.

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Winchester) disk drive, comprising a bit pattern encoding data from an assay of the invention SunOS, Solaris, AIX, SCO Unix, VMS, MV, Macintosh, etc.) floppy diskette or hard (fixed, The invention also preferably provides a magnetic disk, such as an IBM-compatible (DOS, Windows, Windows95/98/2000, Windows NT, OS/2) or other format (e.g., Linux, in a file format suitable for retrieval and processing in a computerized sequence analysis, comparison, or relative quantitation method.

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line, wireless network, optical fiber, or other suitable signal transmission medium, whereby at The invention also provides a network, comprising a plurality of computing devices least one network device (e.g., computer, disk array, etc.) comprises a pattern of magnetic linked via a data link, such as an Ethernet cable (coax or 10BaseT), telephone line, ISDN domains (e.g., magnetic disk) and/or charge domains (e.g., an array of DRAM cells)

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composing a bit pattern encoding data acquired from an assay of the invention.

generating an electronic signal on an electronic communications device, such as a modem, ISDN terminal adapter, DSL, cable modem, ATM switch, or the like, wherein the signal includes (in native or encrypted format) a bit pattern encoding data from an assay or a The invention also provides a method for transmitting assay data that includes

In a preferred embodiment, the invention provides a computer system for comparing a identity and gap weight to the target data. A central processor is preferably initialized to load obtained by the method of the invention, and ranking database targets based on the degree of the computer program results in the central processor retrieving the assay data from the data Data for a query target is entered into the central processor via an I/O device. Execution of database comprising a plurality of assay results obtained by the method of the invention. and execute the computer program for alignment and/or comparison of the assay results. query target to a database containing an array of data structures, such as an assay result file, which comprises a binary description of an assay result. 2

SRAM, SGRAM, SDRAM, EPROM, bubble memory, flash memory, etc.); an I/O device can assay characteristic (e.g., binding to a selected affinity moiety) and the same characteristic of nolecular biology software package (e.g., UWGCG Sequence Analysis Software, Darwin); a SDRAM). Targets are ranked according to the degree of correspondence between a selected The target data or record and the computer program can be transferred to secondary the query target and results are output via an I/O device. For example, a central processor can be a conventional computer (e.g., Intel Pentium, PowerPC, Alpha, PA-8000, SPARC, data file can be an optical or magnetic disk, a data server, a memory device (e.g., DRAM, MIPS 4400, MIPS 10000, VAX, etc.); a program can be a commercial or public domain memory, which is typically random access memory (e.g., DRAM, SRAM, SGRAM, or be a terminal comprising a video display and a keyboard, a modem, an ISDN terminal 15 2 25

which may be stored in the computer; (3) a comparison target, such as a query target; and (4) a program for alignment and comparison, typically with rank-ordering of comparison results collection of peptide sequence specificity records obtained by methods of the inventions, The invention also preferably provides the use of a computer system, e.g., which ypically comprises one or more of: (1) a computer; (2) a stored bit pattern encoding a on the basis of computed similarity values.

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idapter, an Ethernet port, a punched card reader, a magnetic strip reader, or other suitable I/O

# Characteristics of ovarian cancer-associated proteins

proteins, transmembrane proteins, or intracellular proteins. In one embodiment, the ovarian cytoplasm and/or in the nucleus. Intracellular proteins are involved in all aspects of cellular proteins often results in unregulated or disregulated cellular processes. See, e.g., Alberts, et phosphatase activity, protease activity, nucleotide cyclase activity, polymerase activity, and organizing complexes of proteins, or targeting proteins to various subcellular localizations, function and replication (including, e.g., signaling pathways); aberrant expression of such Ovarian cancer proteins of the present invention may be categorized as secreted the like. Intracellular proteins can also serve as docking proteins that are involved in intracellular proteins have enzymatic activity such as protein kinase activity, protein cancer protein is an intracellular protein. Intracellular proteins may be found in the al. (eds. 1994) Molecular Biology of the Cell (3d ed.) Garland. For example, many and are often involved in maintaining the structural integrity of organelles.

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only a few, have been shown to mediate protein-protein interactions. Some of these may also Nuc. Acids Res. 28:263-266; Sonnhammer, et al. (1997) Proteins 28:405-420; Bateman, et al. (1999) Nuc. Acids Res. 27:260-262, and Sonnhammer, et al. (1998) Nuc. Acids Res. 26:320from SH2 domains, also bind tyrosine phosphorylated targets. SH3 domains bind to prolinehe involved in binding to phospholipids or other second messengers. As will be appreciated Center in England, and the Karolinska Institute in Sweden. See, e.g., Bateman, et al. (2000) enzymatic potential of the molecule and/or molecules with which the protein may associate. Versions are available via the internet from Washington University in St. Louis, the Sanger An increasingly appreciated concept in characterizing proteins is the presence in the sequence alignments and hidden Markov models covering many common protein domains. by one of ordinary skill in the art, these motifs can be identified on the basis of amino acid proteins of one or more structural motifs for which defined functions have been attributed. phosphorylated targets in a sequence dependent manner. PTB domains, which are distinct rich targets. In addition, PH domains, tetratricopeptide repeats and WD domains to name In addition to the highly conserved sequences found in the enzymatic domain of proteins, sequence; thus, an analysis of the sequence of proteins may provide insight into both the highly conserved sequences have been identified in proteins that are involved in protein-One useful database is Pfam (protein families), which is a large collection of multiple protein interaction. For example, Src-homology-2 (SH2) domains bind tyrosine-ဗ္က

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WO 02/102235

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kinases have both protein kinase activity and SH2 domains. In addition, autophosphorylation for intracellular proteins. For example, the intracellular domain may have enzymatic activity domains of such proteins may have a number of functions including those already described domain of transmembrane proteins serves both roles. For example certain receptor tyrosine In another preferred embodiment, the ovarian cancer sequences are transmembrane They may have an intracellular domain, an extracellular domain, or both. The intracellular proteins. Transmembrane proteins are molecules that span a phospholipid bilayer of a cell. and/or may serve as a binding site for additional proteins. Frequently the intracellular

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For example, receptor tyrosine kinases, certain cytokine receptors, receptor guanylyl cyclases Transmembrane proteins may contain from one to many transmembrane domains. and receptor serine/threonine protein kinases contain a single transmembrane domain.

of tyrosines on the receptor molecule itself, creates binding sites for additional SH2 domain

containing proteins.

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amino acids. Therefore, upon analysis of the amino acid sequence of a particular protein, the receptors (GPCRs) are classified as "seven transmembrane domain" proteins, as they contain However, various other proteins including channels and adenylyl cyclases contain numerous ransmembrane domains. Many important cell surface receptors such as G protein coupled approximately 17 consecutive hydrophobic amino acids that may be followed by charged 7 membrane spanning regions. Characteristics of transmembrane domains include 15 20

ocalization and number of transmembrane domains within the protein may be predicted (see, include, but are not limited to the insulin receptor, insulin-like growth factor receptor, human growth hormone receptor, glucose transporters, transferrin receptor, epidermal growth factor receptor, low density lipoprotein receptor, epidermal growth factor receptor, leptin receptor, e.g., PSORT web site http://psort.nibb.ac.jp/). Important transmembrane protein receptors interleukin receptors, e.g., IL-1 receptor, IL-2 receptor, etc. 25

The extracellular domains of transmembrane proteins are diverse; however, conserved and/or functions have been ascribed to different extracellular motifs. Many extracellular motifs are found repeatedly among various extracellular domains. Conserved structure

domains are involved in binding to other molecules. In one aspect, extracellular domains are growth factors such as EGF, FGF, and PDGF are circulating growth factors that bind to their found on receptors. Factors that bind the receptor domain include circulating ligands, which may be peptides, proteins, or small molecules such as adenosine and the like. For example, 30

WO 02/102235

cognate receptors to initiate a variety of cellular responses. Other factors include cytokines, mitogenic factors, neurotrophic factors and the like. Extracellular domains also bind to cellassociated molecules, or may be processed or shed to the blood stream. In this respect, they can mediate cell-cell interactions. Cell-associated ligands can be tethered to the cell, e.g., via a glycosylphosphatidylinositol (GPI) anchor, or may themselves be transmembrane proteins. Extracellular domains also associate with the extracellular matrix and contribute to the maintenance of the cell structure.

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Ovarian cancer proteins that are transmembrane are particularly preferred in the present invention as they are readily accessible targets for immunotherapeutics, as are described herein. In addition, as outlined below, transmembrane proteins can be also useful in imaging modalities. Antibodies may be used to label such readily accessible proteins in situ. Alternatively, antibodies can also label intracellular proteins, in which case samples are typically permeablized to provide access to intracellular proteins. In addition, some membrane proteins can be processed to release a soluble protein, or to expose a residual fragment. Released soluble proteins may be useful diagnostic markers, processed residual protein fragments may be useful disease.

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It will also be appreciated by those in the art that a transmembrane protein can be made soluble by removing transmembrane sequences, e.g., through recombinant methods. Furthermore, transmembrane proteins that have been made soluble can be made to be secreted through recombinant means by adding an appropriate signal sequence.

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In another embodiment, the ovarian cancer proteins are secreted proteins; the secretion of which can be either constitutive or regulated. These proteins may have a signal peptide or signal sequence that targets the molecule to the secretory pathway. Secreted proteins are involved in numerous physiological events; e.g., if circulating, they often serve to transmit signals to various other cell types. The secreted protein may function in an autocrine manner (acting on the cell that secreted the factor), a paracrine manner (acting on cells in close proximity to the cell that secreted the factor), an endocrine manner (acting on cells at a distance, e.g., secretion into the blood stream), or exocrine (secretion, e.g., through a duct or to an adjacent epithelial surface as sweat glands, sebaceous glands, pancreatic ducts, 30 lacrimal glands, mammary glands, wax producing glands of the ear, etc.). Thus, secreted molecules often find use in modulating or altering numerous aspects of physiology. Ovarian cancer proteins that are secreted proteins are particularly preferred as good diagnostic markers, e.g., for blood, plasma, serum, or stool tests. Those which are enzymes may be

antibody or small molecule therapeutic targets. Others may be useful as vaccine targets, e.g., via CTL mechanisms, as protein or DNA vaccines.

PCT/US02/19297

#### Use of ovarian cancer nucleic acids

As described above, ovarian cancer sequence is initially identified by substantial nucleic acid and/or amino acid sequence homology or linkage to the ovarian cancer sequences outlined herein. Such homology can be based upon the overall nucleic acid or amino acid sequence, and is generally determined as outlined below, using either homology programs or hybridization conditions. Typically, linked sequences on a mRNA are found on the same molecule.

The ovarian cancer nucleic acid sequences of the invention, e.g., in Table 1-26, can be fragments of larger genes, e.g., they are nucleic acid segments. "Genes" in this context includes coding regions, non-coding regions, and mixtures of coding and non-coding regions. Accordingly, as will be appreciated by those in the art, using the sequences provided herein,

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15 extended sequences, in either direction, of the ovarian cancer genes can be obtained, using techniques well known in the art for cloning either longer sequences or the full length sequences; see Ausubel, et al., supra. Much can be done by informatics and many sequences can be clustered to include multiple sequences corresponding to a single gene, e.g., systems such as UniGene (see, http://www.ncbi.nlm.nih.gov/UniGene/).

Once the ovarian cancer nucleic acid is identified, it can be cloned and, if necessary, its constituent parts recombined to form the entire ovarian cancer nucleic acid coding regions or the entire mRNA sequence. Once isolated from its natural source, e.g., contained within a plasmid or other vector or excised as a linear nucleic acid segment, the recombinant ovarian cancer nucleic acid can be further-used as a probe to identify and isolate other ovarian cancer nucleic acids, e.g., extended coding regions. It can also be used as a "precursor" nucleic acid to make modified or variant ovarian cancer nucleic acids and proteins.

The ovarian cancer nucleic acids of the present invention are useful in several ways. In a first embodiment, nucleic acid probes to the ovarian cancer nucleic acids are made and attached to biochips to be used in screening and diagnostic methods, as outlined below, or for administration, e.g., for gene therapy, vaccine, RNAi, and/or antisense applications. Alternatively, the ovarian cancer nucleic acids that include coding regions of ovarian cancer proteins can be put into expression vectors for the expression of ovarian cancer proteins, again for screening purposes or for administration to a patient.

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In a preferred embodiment, nucleic acid probes to ovarian cancer nucleic acids (both the nucleic acid sequences outlined in the figures and/or the complements thereof) are made. The nucleic acid probes attached to the biochip are designed to be substantially complementary to the ovarian cancer nucleic acids, e.g., the target sequence (either the target sequence of the sample or to other probe sequences, e.g., in sandwich assays), such that hybridization of the target sequence and the probes of the present invention occurs. As outlined below, this complementarity need not be perfect; there may be any number of base pair mismatches which will interfere with hybridization between the target sequence and the single stranded nucleic acids of the present invention. However, if the number of mutations is so great that no hybridization can occur under even the least stringent of hybridization conditions, the sequence is not a complementary target sequence. Thus, by "substantially complementary" herein is meant that the probes are sufficiently complementary to the target sequences to hybridize under normal reaction conditions, particularly high stringency conditions, as outlined herein.

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A nucleic acid probe is generally single stranded but can be partially single and partially double stranded. The strandedness of the probe is dictated by the structure, composition, and properties of the target sequence. In general, the nucleic acid probes range from about 8 to about 100 bases long, with from about 10 to about 80 bases being preferred, and from about 30 to about 50 bases being particularly preferred. That is, generally whole genes are not used. In some embodiments, much longer nucleic acids can be used, up to hundreds of bases.

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In a preferred embodiment, more than one probe per sequence is used, with either overlapping probes or probes to different sections of the target being used. That is, two, three, four or more probes, with three being preferred, are used to build in a redundancy for a particular target. The probes can be overlapping (e.g., have some sequence in common), or separate. In some cases, PCR primers may be used to amplify signal for higher sensitivity.

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As will be appreciated by those in the art, nucleic acids can be attached or immobilized to a solid support in a wide variety of ways. By "immobilized" and grammatical equivalents herein is meant the association or binding between the nucleic acid probe and the solid support is sufficient to be stable under the conditions of binding, washing, analysis, and removal as outlined below. The binding can typically be covalent or non-covalent. By "non-covalent binding" and grammatical equivalents herein is meant one or more of electrostatic, hydrophilic, and bydrophobic interactions. Included in non-covalent binding is the covalent

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WO 02/102135 PCT/US02/19297

attachment of a molecule, such as, streptavidin to the support and the non-covalent binding of the biotinylated probe to the streptavidin. By "covalent binding" and grammatical equivalents herein is meant that the two moieties, the solid support and the probe, are attached by at least one bond, including sigma bonds, pi bonds and coordination bonds.

5 Covalent bonds can be formed directly between the probe and the solid support or can be formed by a cross linker or by inclusion of a specific reactive group on either the solid support or the probe or both molecules. Immobilization may also involve a combination of covalent and non-covalent interactions.

In general, the probes are attached to the biochip in a wide variety of ways, as will be appreciated by those in the art. As described herein, the nucleic acids can either be synthesized first, with subsequent attachment to the biochip, or can be directly synthesized on the biochia.

The biochip comprises a suitable solid substrate. By "substrate" or "solid support" or other grammatical equivalents herein is meant a material that can be modified to contain discrete individual sites appropriate for the attachment or association of the nucleic acid probes and is amenable to at least one detection method. As will be appreciated by those in the art, the number of possible substrates are very large, and include, but are not limited to, glass and modified or functionalized glass, plastics (including acrylics, polystyrene and copolymers of styrene and other materials, polypropylene, polyethylene, polybutylene,

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20 polyurethanes, TeflonJ, etc.), polysaccharides, nylon or nitrocellulose, resins, silica or silicabased materials including silicon and modified silicon, carbon, metals, inorganic glasses, plastics, etc. In general, the substrates allow optical detection and do not appreciably fluoresce. See, e.g., WO0055627 Reusable Low Fluorescent Plastic Biochip.

Generally the substrate is planar, although as will be appreciated by those in the art,

other configurations of substrates may be used as well. For example, the probes may be
placed on the inside surface of a tube, for flow-through sample analysis to minimize sample
volume. Similarly, the substrate may be flexible, such as a flexible foam, including closed
cell foams made of particular plastics.

In a preferred embodiment, the surface of the biochip and the probe may be
derivatized with chemical functional groups for subsequent attachment of the two. Thus, e.g.,
the biochip is derivatized with a chemical functional group including, but not limited to,
amino groups, carboxyl groups, oxo groups and thiol groups, with amino groups being
particularly preferred. Using these functional groups, the probes can be attached using

WO 02/102235 PCT/US02/19297

functional groups on the probes. For example, nucleic acids containing amino groups can be attached to surfaces comprising amino groups, e.g., using linkers as are known in the art; e.g., homo-or hetero-bifunctional linkers as are well known (see 1994 Pierce Chemical Company catalog, technical section on cross-linkers, pages 155-200). In addition, in some cases, additional linkers, such as alkyl groups (including substituted and heteroalkyl groups) may be

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In this embodiment, oligonucleotides are synthesized as is known in the art, and then attached to the surface of the solid support. As will be appreciated by those skilled in the art, either the 5' or 3' terminus may be attached to the solid support, or attachment may be via an internal nucleoside.

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In another embodiment, the immobilization to the solid support may be very strong, yet non-covalent. For example, biotinylated oligonucleotides can be made, which bind to surfaces covalently coated with streptavidin, resulting in attachment.

Alternatively, the oligonucleotides may be synthesized on the surface, as is known in the art. For example, photoactivation techniques utilizing photopolymerization compounds and techniques are used. In a preferred embodiment, the nucleic acids can be synthesized in situ, using well known photolithographic techniques, such as those described in WO 95/25116; WO 95/35505, U.S. Patent Nos. 5,700,637 and 5,445,934; and references cited within, all of which are expressly incorporated by reference; these methods of attachment form the basis of the Affymetrix GeneChipt<sup>TM</sup> technology.

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Often, amplification-based assays are performed to measure the expression level of ovarian cancer-associated sequences. These assays are typically performed in conjunction with reverse transcription. In such assays, an ovarian cancer-associated nucleic acid sequence acts as a template in an amplification reaction (e.g., Polymerase Chain Reaction, or PCR). In a quantitative amplification, the amount of amplification product will be proportional to the amount of template in the original sample. Comparison to appropriate controls provides a measure of the amount of ovarian cancer-associated RNA. Methods of quantitative amplification are well known to those of skill in the art. Detailed protocols for quantitative PCR are available. See, e.g., Innis, et al.(1990) PCR Protocols: A Guide to Methods and Applications Academic Press.

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In some embodiments, a TaqMan based assay is used to measure expression. TaqMan based assays use a fluorogenic oligonucleotide probe that contains a 5' fluorescent dye and a 3' quenching agent. The probe hybridizes to a PCR product, but cannot itself be

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WO 02/102235 PCT/US02/19297

extended due to a blocking agent at the 3' end. When the PCR product is amplified in subsequent cycles, the 5' nuclease activity of the polymerase, e.g., AmpliTaq, results in the cleavage of the TaqMan probe. This cleavage separates the 5' fluorescent dye and the 3' quenching agent, thereby resulting in an increase in fluorescence as a function of

s amplification (see, e.g., literature provided by Perkin-Elmer, e.g., www2.perkin-elmer.com).

Other suitable amplification methods include, but are not limited to, ligase chain reaction (LCR, see Wu and Wallace (1989) <u>Genomics</u> 4:560-569; Landegren, et al. (1988)

<u>Science</u> 241:1077-1980; and Barringer, et al. (1990) <u>Gene</u> 89:117-122), transcription

10 sequence replication (Guatelli, et al. (1990) <u>Proc. Nat'l Acad. Sci. USA</u> 87:1874-1878), dot PCR, linker adapter PCR, etc.

amplification (Kwoh, et al. (1989) Proc. Nat'l Acad. Sci. USA 86:1173-1177), self-sustained

# Expression of ovarian cancer proteins from nucleic acids

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In a preferred embodiment, ovarian cancer nucleic acids, e.g., encoding ovarian cancer proteins are used to make a variety of expression vectors to express ovarian cancer proteins which can then be used in screening assays, as described below. Expression vectors and recombinant DNA technology are well known and are used to express proteins. Sec, e.g., Ausubel, supra; and Fernandez and Hoeffler (eds. 1999) Gene Expression Systems Academic Press. The expression vectors may be either self-replicating extrachromosomal vectors or

vectors which integrate into a host genome. Generally, these expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the nucleic acid encoding the ovarian cancer protein. The term "control sequences" refers to DNA sequences used for the expression of an operably linked coding sequence in a particular host organism. Control sequences that are suitable for prokaryotes, e.g., include a promoter, optionally an

25 operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a pre-sequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a pre-protein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation; and two sequences may be operably linked when they are physically part of the same polymer. Generally,

"operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is typically accomplished by ligation at convenient restriction sites. If such sites do not exist, synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice. Transcriptional and translational regulatory nucleic acid will generally be appropriate to the host cell used to express the ovarian cancer protein. Numerous types of appropriate expression vectors, and suitable regulatory sequences are known in the art for a variety of host cells.

In general, transcriptional and translational regulatory sequences may include, but are not limited to, promoter sequences, ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator sequences. In a preferred embodiment, the regulatory sequences include a promoter and transcriptional start and stop sequences.

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Promoter sequences typically encode constitutive or inducible promoters. The promoters may be naturally occurring promoters or hybrid promoters. Hybrid promoters, which combine elements of more than one promoter, are also known in the art, and are useful in the present invention.

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In addition, an expression vector may comprise additional elements. For example, the expression vector may have two replication systems, thus allowing it to be maintained in two organisms, e.g., in mammalian or insect cells for expression and in a procaryotic host for cloning and amplification. Furthermore, for integrating expression vectors, the expression vector contains at least one sequence homologous to the host cell genome, and preferably two homologous sequences which flank the expression construct. The integrating vector may be directed to a specific locus in the host cell by selecting the appropriate homologous sequence for inclusion in the vector. Constructs for integrating vectors are available. See, e.g.,

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In addition, in a preferred embodiment, the expression vector contains a selectable marker gene to allow the selection of transformed host cells. Selection genes are well known in the art and will vary with the host cell used.

The ovarian cancer proteins of the present invention are produced by culturing a host cell transformed with an expression vector containing nucleic acid encoding an ovarian cancer protein, under the appropriate conditions to induce or cause expression of the ovarian cancer protein. Conditions appropriate for ovarian cancer protein expression will vary with

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WO 02/102235 PCT/US02/19297

the choice of the expression vector and the host cell, and will be easily ascertained by one skilled in the art through routine experimentation or optimization. For example, the use of constitutive promoters in the expression vector will require optimizing the growth and proliferation of the host cell, while the use of an inducible promoter requires the appropriate growth conditions for induction. In addition, in some embodiments, the timing of the harvest is important. For example, the baculovirus systems used in insect cell expression are lytic viruses, and thus harvest time selection can be crucial for product yield.

Appropriate host cells include yeast, bacteria, archaebacteria, fungi, and insect and animal cells, including mammalian cells. Of particular interest are Saccharomyces cerevisiae and other yeasts, B. coli, Bacillus subtilis, Sf9 cells, C129 cells, 293 cells, Neurospora, BHK, CHO, COS, HeLa cells, HUVEC (human umbilical vein endothelial cells), THP1 cells (a macrophage cell line) and various other human cells and cell lines.

In a preferred embodiment, the ovarian cancer, proteins are expressed in mammalian cells. Mammalian expression systems are also known in the art, and include retroviral and adenoviral systems. One expression vector system is a retroviral vector system such as is generally described in PCT/US97/01019 and PCT/US97/01048, both of which are hereby expressly incorporated by reference. Of particular use as mammalian promoters are the promoters from mammalian viral genes, since the viral genes are often highly expressed and have a broad host range. Examples include the SV40 early promoter, mouse mammary tumor

virus LTR promoter, adenovirus major late promoter, herpes simplex virus promoter, and the CMV promoter. See, e.g., Fernandez and Hoeffler, supra. Typically, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. Examples of transcription terminator and polyadenylation signals include those derived form SV40.

The methods of introducing exogenous nucleic acid into mammalian hosts, as well as other hosts, is well known in the art, and will vary with the host cell used. Techniques include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, viral infection, encapsulation of the

polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

In a preferred embodiment, ovarian cancer proteins are expressed in bacterial systems.

Bacterial expression systems are well known in the art. Promoters from bacteriophage may also be used and are known in the art. In addition, synthetic promoters and hybrid promoters

WO 02/102235

PCT/US02/19297

are also useful; e.g., the tac promoter is a hybrid of the trp and lac promoter sequences.

Furthermore, a bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription. In addition to a functioning promoter sequence, an efficient ribosome binding site is desirable.

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The expression vector may also include a signal peptide sequence that provides for secretion of the ovarian cancer protein in bacteria. The protein is either secreted into the growth media (gram-positive bacteria) or into the periplasmic space, located between the inner and outer membrane of the cell (gram-negative bacteria). The bacterial expression vector may also include a selectable marker gene to allow for the selection of bacterial strains that have been transformed. Suitable selection genes include genes which render the bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin, neomycin, and tetracycline. Selectable markers also include biosynthetic genes, such as those in the histidine, tryptophan, and leucine biosynthetic pathways. These components are assembled into expression vectors. Expression vectors for bacteria are well known in the art, and include vectors for Bacillus subtilis, B. coli, Streptococcus cremoris, and Streptococcus lividans, among others. See Fernandez and Hoeffler, supra. The bacterial expression vectors are transformed into bacterial host cells using techniques well known in the art, such as calcium chloride treatment, electroporation, and others.

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In one embodiment, ovarian cancer proteins are produced in insect cells. Expression vectors for the transformation of insect cells, and in particular, baculovirus-based expression vectors, are well known in the art.

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In a preferred embodiment, an ovarian cancer protein is produced in yeast cells. Yeast expression systems are well known in the art, and include expression vectors for Saccharomyces cerevisiae, Candida albicans and C. maltosa, Hansenula polymorpha, Kluyveromyces fragilis and K. lactis, Pichia guillerimondii and P. pastoris, Schizosaccharomyces pombe, and Yarrowia lipolytica.

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The ovarian cancer protein may also be made as a fusion protein, using techniques well known in the art. Thus, e.g., for the creation of monoclonal antibodies, if the desired epitope is small, the ovarian cancer protein may be fused to a carrier protein to form an immunogen. Alternatively, the ovarian cancer protein may be made as a fusion protein to increase expression, or for other reasons. For example, when the ovarian cancer protein is an ovarian cancer peptide, the nucleic acid encoding the peptide may be linked to other nucleic acid for expression purposes.

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In a preferred embodinnent, the ovarian cancer protein is purified or isolated after expression. Ovarian cancer proteins may be isolated or purified in a variety of ways known to those skilled in the art depending on what other components are present in the sample. Standard purification methods include electrophoretic, molecular, immunological and

chromatographic techniques, including ion exchange, hydrophobic, affinity, and reversephase HPLC chromatography, and chromatofocusing. For example, the ovarian cancer
protein may be purified using a standard anti-ovarian cancer protein antibody column.
Ultrafiltration and diafiltration techniques, in conjunction with protein concentration, are also
useful. For general guidance in suitable purification techniques, see Scopes (1982) <u>Protein</u>
10 <u>Purification</u> Springer-Verlag, The degree of purification necessary will vary depending on

Once expressed and purified if necessary, the ovarian cancer proteins and nucleic acids are useful in a number of applications. They may be used as immunoselection reagents, as vaccine reagents, as screening agents, etc.

the use of the ovarian cancer protein. In some instances no purification will be necessary.

Variants of ovarian cancer proteins

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In one embodiment, the ovarian cancer proteins are derivative or variant ovarian cancer proteins as compared to the wild-type sequence. That is, as outlined more fully below, the derivative ovarian cancer peptide will often contain at least one amino acid substitution, deletion or insertion, with amino acid substitutions being particularly preferred. The amino acid substitution, insertion, or deletion may occur at most any residue within the ovarian cancer peptide.

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Also included within one embodiment of ovarian cancer proteins of the present invention are amino acid sequence variants. These variants typically fall into one or more of three classes: substitutional, insertional or deletional variants. These variants ordinarily are prepared by site specific mutagenesis of nucleotides in the DNA encoding the ovarian cancer protein, using cassette or PCR mutagenesis or other techniques well known in the art, to produce DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture as outlined above. However, variant ovarian cancer protein fragments having up to about 100-150 residues may be prepared by in vitro synthesis using established techniques. Amino acid sequence variants are characterized by the predetermined nature of the variation,

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ovarian cancer protein amino acid sequence. The variants typically exhibit the same

a feature that sets them apart from naturally occurring allelic or interspecies variation of the

WO 02/102235

PCT/US02/19297

qualitative biological activity as the naturally occurring analogue, although variants can also be selected which have modified characteristics as will be more fully outlined below.

conducted at the target codon or region and the expressed ovarian cancer variants screened mutations at predetermined sites in DNA having a known sequence are well known, e.g., predetermined, the mutation per se need not be predetermined. For example, in order to M13 primer mutagenesis and PCR mutagenesis. Screening of the mutants is done using optimize the performance of a mutation at a given site, random mutagenesis may be for the optimal combination of desired activity. Techniques for making substitution While the site or region for introducing an amino acid sequence variation is assays of ovarian cancer protein activities. Amino acid substitutions are typically of single residues; insertions usually will be on the order of from about 1 to 20 amino acids, although considerably larger insertions may be tolerated. Deletions range from about 1 to about 20 residues, although in some cases deletions may be much larger.

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circumstances. When small alterations in the characteristics of the ovarian cancer protein are at a final derivative. Generally these changes are done on a few amino acids to minimize the Substitutions, deletions, insertions or any combination thereof may be used to arrive desired, substitutions are generally made in accordance with the amino acid substitution alteration of the molecule. However, larger changes may be tolerated in certain relationships provided in the definition section.

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selected to modify the characteristics of the ovarian cancer proteins as needed. Alternatively, the variant may be designed such that the biological activity of the ovarian cancer protein is The variants typically exhibit the same qualitative biological activity and will elicit the same immune response as the naturally-occurring analog, although variants also are altered. For example, glycosylation sites may be altered or removed.

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polypeptide's properties are those in which (a) a hydrophilic residue, e.g., serine or threonine the charge or hydrophobicity of the molecule at the target site; or the bulk of the side chain. substitutions may be made which more significantly affect: the structure of the polypeptide backbone in the area of the alteration, for example the alpha-helical or beta-sheet structure; Substantial changes in function or immunological identity are made by selecting is substituted for (or by) a hydrophobic residue, e.g., leucine, isoleucine, phenylalanine, The substitutions which in general are expected to produce the greatest changes in the substitutions that are less conservative than those described above. For example,

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residue having an electropositive side chain, e.g., lysine, arginine, or histidine, is substituted for (or by) an electronegative residue, e.g., glutamic or aspartic acid; (d) a residue having a valine, or alanine; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain,

e.g., glycine; or (e) a proline residue is incorporated or substituted, which changes the degree of rotational freedom of the peptidyl bond.

residues of an ovarian cancer polypeptide with an organic derivatizing agent that is capable of Covalent modifications of ovarian cancer polypeptides are included within the scope of this invention. One type of covalent modification includes reacting targeted amino acid

- polypeptide. Derivatization with bifunctional agents is useful, for instance, for crosslinking nethod for purifying anti-ovarian cancer polypeptide antibodies or screening assays, as is ovarian cancer polypeptides to a water-insoluble support matrix or surface for use in the reacting with selected side chains or the N-or C-terminal residues of an ovarian cancer more fully described below. Commonly used crosslinking agents include, e.g., 1,1-2
  - bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, e.g., esters maleimido-1,8-octane and agents such as methyl-3-((p-azidophenyl)dithio)propioimidate. with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), bifunctional maleimides such as bis-N-13
- corresponding glutamic and aspartic acid residues, respectively, hydroxylation of proline and Other modifications include deamidation of glutamine and asparagine residues to the 79-86, Creighton (1983) Proteins: Structure and Molecular Properties Freeman), acetylation methylation of the amino groups of the lysine, arginine, and histidine side chains (e.g., pp. lysine, phosphorylation of hydroxyl groups of serine, threonine, or tyrosine residues, of the N-terminal amine, and amidation of a C-terminal carboxyl group. 2
- polypeptide, and/or adding one or more glycosylation sites that are not present in the native within the scope of this invention comprises altering the native glycosylation pattern of the polypeptide. "Altering the native glycosylation pattern" is intended for purposes herein to sequence ovarian cancer polypeptide. Glycosylation patterns can be altered in many ways. mean deleting one or more carbohydrate moieties found in native sequence ovarian cancer For example the use of different cell types to express ovarian cancer-associated sequences Another type of covalent modification of the ovarian cancer polypeptide included can result in different glycosylation patterns. 3 22

Addition of glycosylation sites to ovarian cancer polypeptides may also be

accomplished by altering the amino acid sequence thereof. The alteration may be made, e.g., by the addition of, or substitution by, one or more serine or threonine residues to the native sequence ovarian cancer polypeptide (for O-linked glycosylation sites). The ovarian cancer amino acid sequence may optionally be altered through changes at the DNA level, particularly by mutating the DNA encoding the ovarian cancer polypeptide at pre-selected bases such that codons are generated that will translate into the desired amino acids.

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Another means of increasing the number of carbohydrate moieties on the ovarian cancer polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. See, e.g., WO 87/05330, and pp. 259-306 in Aplin and Wriston (1981) CRC Crit. Rev. Biochem, CRC Press.

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Removal of carbohydrate moieties present on the ovarian cancer polypeptide may be accomplished chemically or enzymatically or by mutational substitution of codons encoding for amino acid residues that serve as targets for glycosylation. Chemical deglycosylation techniques are applicable. See, e.g., Sojar and Bahl (1987) <u>Arch. Biochem. Biophys.</u> 259:52-57; and Edge, et al. (1981) <u>Anal. Biochem.</u> 118:131-137. Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo-and exo-glycosidases. See, e.g., Thotakura, et al. (1987) <u>Meth. Enzymol.</u>, 138:350-359.

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Another type of covalent modification of ovarian cancer comprises linking the ovarian cancer polypeptide to one of a variety of non-proteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol, or polyoxyalkylene. See, e.g., U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192; or 4,179,337.

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Ovarian cancer polypeptides of the present invention may also be modified in a way to form chimeric molecules, e.g., comprising an ovarian cancer polypeptide fused to another heterologous polypeptide or amino acid sequence. In one embodiment, such a chimeric molecule comprises a fusion of an ovarian cancer polypeptide with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino-or carboxyl-terminus of the ovarian cancer polypeptide. The presence of such epitope-tagged forms of an ovarian cancer polypeptide can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag enables the ovarian cancer polypeptide to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag. In an alternative embodiment, the chimetic molecule may comprise a fusion of an ovarian cancer polypeptide with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of

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WO 02/102235 PCT/US02/19297

the chimeric molecule, such a fusion could be to the Fc region of an IgG molecule.

Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; His6 and metal chelation tags, the flu HA tag polypeptide and its antibody 12CA5 (Field, et al.

- 5 (1988) Mol. Cell. Biol. 8:2159-2165); the e-myc tag and the 8F9, 3C7, 6E10, G4, B7, and 9E10 antibodies thereto (Evan, et al. (1985) Mol. Cell. Biol. 5:3610-3616); and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody (Paborsky, et al. (1990) Protein Engineering 3:547-553). Other tag polypeptides include, e.g., the Flag-peptide (Hopp, et al. (1988) BioTechnology 6:1204-1210); the KT3 epitope peptide (Martin, et al. (1992) Science
- 10 255:192-194); tubulin epitope peptide (Skinner, et al. (1991) <u>J. Biol. Chem.</u> 266:15163-15166); and the T7 gene 10 protein peptide tag (Lutz-Freyermuth et al. (1990) <u>Proc. Nat'l</u> <u>Acad. Sci. USA</u> 87:6393-6397).

Also included are other ovarian cancer proteins of the ovarian cancer family, and ovarian cancer proteins from other organisms, which are cloned and expressed as outlined below. Thus, probe or degenerate polymerase chain reaction (PCR) primer sequences may be used to find other related ovarian cancer proteins from humans or other organisms. As will be appreciated by those in the art, particularly useful probe and/or PCR primer sequences include the unique areas of the ovarian cancer nucleic acid sequence. As is generally known in the art, preferred PCR primers are from about 15 to about 35 nucleotides in length, with

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20 from about 20 to about 30 being preferred, and may contain inosine as needed. The conditions for the PCR reaction are well known in the art (e.g., Innis, <u>PCR Protocols</u>, supra).

## Antibodies to ovarian cancer proteins

In a preferred embodiment, when the ovarian cancer protein is to be used to generate antibodies, e.g., for immunotherapy or immunodiagnosis, the ovarian cancer protein should share at least one epitope or determinant with the full length protein. By "epitope" or "determinant" herein is typically meant a portion of a protein which will generate and/or bind an antibody or T-cell receptor in the context of MHC. Thus, in most instances, antibodies made to a smaller ovarian cancer protein will be able to bind to the full-length protein,

30 particularly linear epitopes. In a preferred embodiment, the epitope is unique; that is, antibodies generated to a unique epitope show little or no cross-reactivity.

Methods of preparing polyclonal antibodies are known to the skilled artisan (e.g., Coligan, supra; and Harlow and Lane, supra). Polyclonal antibodies can be raised in a

WO 02/102235

PCT/US02/19297

mammal, e.g., by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include a protein encoded by a nucleic acid of the figures or fragment thereof or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the manumal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, scrum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

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medium that preferably contains one or more substances that inhibit the growth or survival of thereof, or a fusion protein thereof. Generally, either peripheral blood lymphocytes ("PBLs") <u>Practice</u> Academic Press). Immortalized cell lines are usually transformed mammalian cells, The antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies (1975) Nature 256:495-497. In a hybridoma method, a mouse, hamster, or other appropriate mycloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture produce or are capable of producing antibodies that will specifically bind to the immunizing may be prepared using hybridoma methods, such as those described by Kohler and Milstein for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT are used if cells of human origin are desired, or spleen cells or lymph node cells are used if hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium agent. Alternatively, the lymphocytes may be immunized in vitro. The immunizing agent immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (e.g., pp. 59-103 in Goding (1986) Monoclonal Antibodies: Principles and will typically include a polypeptide encoded by a nucleic acid of Tables 1-26 or fragment host animal, is typically immunized with an immunizing agent to elicit lymphocytes that particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse non-human mammalian sources are desired. The lymphocytes are then fused with an the unfused, immortalized cells. For example, if the parental cells lack the enzyme medium"), which substances prevent the growth of HGPRT-deficient cells.

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In one embodiment, the antibodies are bispecific antibodies. Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at

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least two different antigens or that have binding specificities for two epitopes on the same antigen. In one embodiment, one of the binding specificities is for a protein encoded by a nucleic acid Table 1-26 or a fragment thereof, the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit, preferably one that is

umor specific. Alternatively, tetramer-type technology may create multivalent reagents.

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In a preferred embodiment, the antibodies to ovarian cancer protein are capable of reducing or eliminating a biological function of an ovarian cancer protein, as is described below. That is, the addition of anti-ovarian cancer protein antibodies (either polyclonal or preferably monoclonal) to ovarian cancer tissue (or cells containing ovarian cancer) may reduce or eliminate the ovarian cancer. Generally, at least a 25% decrease in activity, growth, size or the like is preferred, with at least about 50% being particularly preferred and about a 95-100% decrease being especially preferred.

In a preferred embodiment the antibodies to the ovarian cancer proteins are humanized antibodies (e.g., Xenerex Biosciences; Medarex, Inc.; Abgenix, Inc.; Protein Design Labs, Inc.) Humanized forms of non-human (e.g., murine) antibodies are chimeric molecules of immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab, 7 (ab')2 or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary

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determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin

immunoglobulin consensus sequence. The humanized antibody optimally also will comprise
at least a portion of an immunoglobulin constant region (Fe), typically that of a human
immunoglobulin. Humanization can be essentially performed following the method of
Winter and co-workers, e.g., by substituting rodent CDRs or CDR sequences for the

and all or substantially all of the framework (FR) regions are those of a human

WO 02/102235

PCT/US02/19297

corresponding sequences of a human antibody. See, e.g., Jones, et al. (1986) <u>Nature</u> 321:522-525; Riechmann, et al. (1988) <u>Nature</u> 332:323-329; Presta (1992) <u>Curr. Op. Struct. Biol.</u> 2:593-596; and Verhoeyen, et al. (1988) <u>Soience</u> 239:1534-1536). Accordingly, such humanized antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries (see, e.g., Hoogenboom and Winter (1991) I.Mol. Biol. 227:381-388; and Marks, et al. (1991) I.Mol. Biol. 222:381-597) or human monoclonal antibodies (see, e.g., p. 77, Cole, et al. in Reisfeld and Sell (1985) Monoclonal Antibodies and Boerner, et al. (1991) I.Immunol. 147:86-95). Similarly, human antibodies can be made by introducing of human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. See, e.g., U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016; Marks, et al. (1994) Nature 368:812-13; Neuberger (1996) Nature Biotechnology 14:845-81; and Lonberg and Huszar (1995) Intern. Rev. Immunol. 13:65-93.

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By immunotherapy is meant treatment of ovarian cancer, e.g., with an antibody raised against ovarian cancer proteins. As used herein, immunotherapy can be passive or active. Passive immunotherapy as defined herein is the passive transfer of antibody to a recipient (patient). Active immunization is the induction of antibody and/or T-cell responses in a recipient (patient). Induction of an immune response is the result of providing the recipient with an antigen to which antibodies are raised. The antigen may be provided by injecting a polypeptide against which antibodies are desired to be raised into a recipient, or contacting the recipient with a nucleic acid capable of expressing the antigen and under conditions for expression of the antigen, leading to an immune response.

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In a preferred embodiment the ovarian cancer proteins against which antibodies are raised are secreted proteins as described above. Without being bound by theory, antibodies used for treatment, bind and prevent the secreted protein from binding to its receptor, thereby

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inactivating the secreted ovarian cancer protein.

In another preferred embodiment, the ovarian cancer protein to which antibodies are raised is a transmembrane protein. Without being bound by theory, antibodies used for treatment, bind the extracellular domain of the ovarian cancer protein and prevent it from

- binding to other proteins, such as circulating ligands or cell-associated molecules. The antibody may cause down-regulation of the transmembrane ovarian cancer protein. As will be appreciated by one of ordinary skill in the art, the antibody may be a competitive, non-competitive or uncompetitive inhibitor of protein binding to the extracellular domain of the ovarian cancer protein. The antibody is also an antagonist of the ovarian cancer protein.
- 10 Further, the antibody prevents activation of the transmembrane ovarian cancer protein. In one aspect, when the antibody prevents the binding of other molecules to the ovarian cancer protein, the antibody prevents growth of the cell. The antibody may also be used to target or sensitize the cell to cytotoxic agents, including, but not limited to TNF-α, TNF-β, IL-1, INF-γ, and IL-2, or chemotherapeutic agents including 5FU, vinblastine, actinomycin D, cisplatin, methotrexate, and the like. In some instances the antibody belongs to a sub-type that activates serum complement when complexed with the transmembrane protein thereby mediating cytotoxicity or antigen-dependent cytotoxicity (ADCC). Thus, ovarian cancer is treated by administering to a patient antibodies directed against the transmembrane ovarian cancer protein. Antibody-labeling may activate a co-toxin, localize a toxin payload, or

In another preferred embodiment, the antibody is conjugated to an effector moiety. The effector moiety can be any number of molecules, including labeling moieties such as radioactive labels or fluorescent labels, or can be a therapeutic moiety. In one aspect the therapeutic moiety is a small molecule that modulates the activity of the ovarian cancer protein. In another aspect the therapeutic moiety modulates the activity of molecules associated with or in close proximity to the ovarian cancer protein. The therapeutic moiety may inhibit enzymatic activity such as protease or collagenase or protein kinase activity associated with ovarian cancer.

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otherwise provide means to locally ablate cells.

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In a preferred embodiment, the therapeutic moiety can also be a cytotoxic agent. In this method, targeting the cytotoxic agent to ovarian cancer tissue or cells, results in a reduction in the number of afflicted cells, thereby reducing symptoms associated with ovarian cancer. Cytotoxic agents are numerous and varied and include, but are not limited to,

PCT/US02/19297

WO 02/102235

PCT/US02/19297

corresponding fragments include diphtheria A chain, exotoxin A chain, ricin A chain, abrin A proteins, or binding of a radionuclide to a chelating agent that has been covalently attached to radiochemicals made by conjugating radioisotopes to antibodies raised against ovarian cancer the antibody. Targeting the therapeutic moiety to transmembrane ovarian cancer proteins not afflicted area, but also serves to reduce deleterious side effects that may be associated with only serves to increase the local concentration of therapeutic moiety in the ovarian cancer chain, curcin, crotin, phenomycin, enomycin and the like. Cytotoxic agents also include cytotoxic drugs or toxins or active fragments of such toxins. Suitable toxins and their the untargeted therapeutic moiety.

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endocytosis. In another embodiment, a nucleic acid encoding the antibody is administered to the individual or cell. Moreover, wherein the ovarian cancer protein can be targeted within a cell, e.g., the nucleus, an antibody thereto contains a signal for that target localization, e.g., a antibodies are raised is an intracellular protein. In this case, the antibody may be conjugated to a protein which facilitates entry into the cell. In one case, the antibody enters the cell by In another preferred embodiment, the ovarian cancer protein against which the nuclear localization signal.

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proteins. By "specifically bind" herein is meant that the antibodies bind to the protein with a Kd of at least about 0.1 mM, more usually at least about 1 μM, preferably at least about 0.1 The ovarian cancer antibodies of the invention specifically bind to ovarian cancer µM or better, and most preferably, 0.01 µM or better. Selectivity of binding is also

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Detection of ovarian cancer sequence for diagnostic and therapeutic applications

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reflective of the state of the cell. By comparing expression profiles of cells in different states, cell. While two states may have any particular gene similarly expressed, the evaluation of a cellular states in the ovarian cancer phenotype. Expression levels of genes in normal tissue malignant disease are evaluated to provide expression profiles. An expression profile of a particular cell state or point of development is essentially a "fingerprint" of the state of the number of genes simultaneously allows the generation of a gene expression profile that is varying severities of ovarian cancer that relate to prognosis, as outlined below, or in non-(e.g., not undergoing ovarian cancer) and in ovarian cancer tissue (and in some cases, for In one aspect, the RNA expression levels of genes are determined for different

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information regarding which genes are important (including both up- and down-regulation of genes) in each of these states is obtained. Then, diagnosis may be performed or confirmed to determine whether a tissue sample has the gene expression profile of normal or cancerous issue. This will provide for molecular diagnosis of related conditions.

normal versus ovarian cancer tissue. Genes may be turned on or turned off in a particular qualitatively have its expression altered, including an activation or inactivation, in, e.g., qualitative or quantitative differences in the temporal and/or cellular gene expression 'Differential expression," or grammatical equivalents as used herein, refers to patterns within and among cells and tissue. Thus, a differentially expressed gene can

which is detectable by standard techniques. Some genes will be expressed in one state or cell ype, but not in both. Alternatively, the difference in expression may be quantitative, e.g., in qualitatively regulated gene will exhibit an expression pattern within a state or cell type that expression is modulated, either up-regulated, resulting in an increased amount of state, relative to another state thus permitting comparison of two or more states. A ٥,

which expression differs need only be large enough to quantify via standard characterization techniques as outlined below, such as by use of Affymetrix GeneChip<sup>TM</sup> expression arrays. See, e.g., Lockhart (1996) Nature Biotechnology 14:1675-1680. Other techniques include, out are not limited to, quantitative reverse transcriptase PCR, northern analysis, and RNase transcript, or down-regulated, resulting in a decreased amount of transcript. The degree to 12

down-regulation) is at least about 50%, more preferably at least about 100%, more preferably at least about 150%, more preferably at least about 200%, with from 300 to at least 1000% protection. As outlined above, preferably the change in expression (e.g., up-regulation or seing especially preferred. 2

expression may be monitored using nucleic acid probes to the DNA or RNA equivalent of the Evaluation may be at the gene transcript, or the protein level. The amount of gene spectroscopy assays, 2D gel electrophoresis assays, etc. Proteins corresponding to ovarian gene transcript, and the quantification of gene expression levels, or, alternatively, the final gene product itself (protein) can be monitored, e.g., with antibodies to the ovarian cancer protein and standard immunoassays (ELISAs, etc.) or other techniques, including mass

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embodiment, gene expression monitoring is performed simultaneously on a number of genes. cancer genes, e.g., those identified as being important in an ovarian cancer or disease Multiple protein expression monitoring can be performed, or on an individual basis. phenotype, can be evaluated in an ovarian disease diagnostic test. In a preferred 2

PCT/US02/19297 WO 02/102235

In this embodiment, the ovarian cancer nucleic acid probes are attached to biochips as particular sample. The assays are further described below in the example. PCR techniques outlined herein for the detection and quantification of ovarian cancer sequences in a can be used to provide greater sensitivity.

detected. Although DNA or RNA encoding the ovarian cancer protein may be detected, of digoxygenin labeled riboprobe (RNA probe) that is complementary to the mRNA encoding oligonucleotides, cDNA or RNA. Probes also should contain a detectable label, as defined probe for sufficient time to allow the probe to hybridize with the target mRNA. Following examined on a solid support such as nylon membranes and hybridizing the probe with the washing to remove the non-specifically bound probe, the label is detected. For example a an ovarian cancer protein is detected by binding the digoxygenin with an anti-digoxygenin detected. In another method detection of the mRNA is performed in situ. In this method permeabilized cells or tissue samples are contacted with a detectably labeled nucleic acid In a preferred embodiment nucleic acids encoding the ovarian cancer protein are particular interest are methods wherein an mRNA encoding an ovarian cancer protein is secondary antibody and developed with nitro blue tetrazolium and 5-bromo-4-chloro-3herein. In one method the mRNA is detected after immobilizing the nucleic acid to be sample. Following washing to remove the non-specifically bound probe, the label is complementary to and hybridizes with the mRNA and includes, but is not limited to, detected. Probes to detect mRNA can be a nucleotide/deoxynucleotide probe that is indoyl phosphate. 20

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containing ovarian cancer sequences are used in diagnostic assays. This can be performed on described herein (secreted, transmembrane or intracellular proteins) are used in diagnostic assays. The ovarian cancer proteins, antibodies, nucleic acids, modified proteins and cells In a preferred embodiment, various proteins from the three classes of proteins as an individual gene or corresponding polypeptide level. In a preferred embodiment, the expression profiles are used, preferably in conjunction with high throughput screening techniques to allow monitoring for expression profile genes and/or corresponding polypeptides.

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transmembrane, or secreted proteins, find use as prognostic or diagnostic markers of ovarian diagnosis, or prognosis of ovarian disease, and for selection of therapeutic strategy. In one disease. Detection of these proteins in putative ovarian cancer tissue allows for detection, As described and defined herein, ovarian cancer proteins, including intracellular, 2

PCT/US02/19297 WO 02/102235

reducing protein gel, but may be another type of gel, including isoelectric focusing gels and the like). Following separation of proteins, the ovarian cancer protein is detected, e.g., by separates proteins from a sample by electrophoresis on a gel (typically a denaturing and embodiment, antibodies are used to detect ovarian cancer proteins. A preferred method immunoblotting with antibodies raised against the ovarian cancer protein. Methods of immunoblotting are well known to those of ordinary skill in the art.

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Biology: Antibodies in Cell Biology (vol. 37) Academic Press. Cells are contacted with from letectable label. This method finds particular use in simultaneous screening for a plurality of ovarian cancer proteins. As will be appreciated by one of ordinary skill in the art, many other embodiment the antibody is detected by incubating with a secondary antibody that contains a one to many antibodies to the ovarian cancer protein(s). Following washing to remove non-In another preferred method, antibodies to the ovarian cancer protein find use in in detectable label. In another method the primary antibody to the ovarian cancer protein(s) contains a detectable label, e.g., an enzyme marker that can act on a substrate. In another specific antibody binding, the presence of the antibody or antibodies is detected. In one preferred embodiment each one of multiple primary antibodies contains a distinct and situ imaging techniques, e.g., in histology. See, e.g., Asai (ed. 1993) Methods in Cell histological imaging techniques are also provided by the invention. 2 2

In a preferred embodiment the label is detected in a fluorometer which has the ability to detect and distinguish emissions of different wavelengths. In addition, a fluorescence activated cell sorter (FACS) can be used in the method.

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samples to be probed or tested for the presence of ovarian cancer proteins. Antibodies can be from blood, serum, plasma, stool, and other samples. Such samples, therefore, are useful as In another preferred embodiment, antibodies find use in diagnosing ovarian cancer used to detect an ovarian cancer protein by previously described immunoassay techniques technology, and the like. Conversely, the presence of antibodies may indicate an immunc including ELISA, immunoblotting (western blotting), immunoprecipitation, BIACORB response against an endogenous ovarian cancer protein. 23

he skilled artisan can make a diagnosis, a prognosis, or a prediction based on the findings. It acid probes to tissue arrays is done. For example, arrays of tissue samples, including ovarian cancer tissue and/or normal tissue, are made. In situ hybridization (see, e.g., Ausubel, supra) is then performed. When comparing the fingerprints between an individual and a standard, In a preferred embodiment, in situ hybridization of labeled ovarian cancer nucleic ဓ္က

WO 02/102235 PCT/US02/19297

is further understood that the genes which indicate the diagnosis may differ from those which indicate the prognosis and molecular profiling of the condition of the cells may lead to distinctions between responsive or refractory conditions or may be predictive of outcomes.

In a preferred embodiment, the ovarian cancer proteins, antibodies, nucleic acids, modified proteins and cells containing ovarian cancer sequences are used in prognosis assays. As above, gene expression profiles can be generated that correlate to ovarian cancer, clinical, pathological, or other information, in terms of long term prognosis. Again, this may be done on either a protein or gene level, with the use of a plurality of genes being preferred. As above, ovarian cancer probes may be attached to biochips for the detection and quantification of ovarian cancer sequences in a tissue or patient. The assays proceed as outlined above for diagnosis. PCR method may provide more sensitive and accurate quantification.

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#### Assays for therapeutic compounds

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In a preferred embodiment members of the proteins, nucleic acids, and antibodies as described herein are used in drug screening assays. The ovarian cancer proteins, antibodies, nucleic acids, modified proteins and cells containing ovarian cancer sequences are used in drug screening assays or by evaluating the effect of drug candidates on a "gene expression profile" or expression profile of polypeptides. In a preferred embodiment, the expression profiles are used, preferably in conjunction with high throughput screening techniques to allow monitoring for expression profile genes after treatment with a candidate agent. See, e.g., Zlokarnik, et al. (1998) Science 279:84-88; and Heid (1996) Genome Res. 6:986-994.

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In a preferred embodiment, the ovarian cancer proteins, antibodies, nucleic acids, modified proteins and cells containing the native or modified ovarian cancer proteins are used in screening assays. That is, the present invention provides novel methods for screening for compositions which modulate the ovarian cancer phenotype or an identified physiological function of an ovarian cancer protein. As above, this can be done on an individual gene level or by evaluating the effect of drug candidates on a "gene expression profile". In a preferred embodiment, the expression profiles are used, preferably in conjunction with high throughput screening techniques to allow monitoring for expression profile genes after treatment with a candidate agent. See, e.g., Zlokarnik, supra.

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Having identified the differentially expressed genes herein, a variety of assays may be executed. In a preferred embodiment, assays may be run on an individual gene or protein level. That is, having identified a particular gene as up regulated in ovarian cancer, test

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WO 02/102235 PCT/US02/19297

compounds can be screened for the ability to modulate gene expression or for binding to the ovarian cancer protein. "Modulation" thus includes both an increase and a decrease in gene expression. The preferred amount of modulation will depend on the original change of the gene expression in normal versus tissue undergoing ovarian cancer, with changes of at least 10%, preferably 50%, more preferably 100-300%, and in some embodiments 300-1000% or greater. Thus, if a gene exhibits a 4-fold increase in ovarian cancer tissue compared to normal tissue, a decrease of about four-fold is often desired; similarly, a 10-fold decrease in ovarian cancer tissue compared to normal tissue often provides a target value of a 10-fold increase in expression to be induced by the test compound.

quantification of gene expression may be monitored using nucleic acid probes and the quantification of gene expression levels, or, alternatively, the gene product itself can be monitored, e.g., through the use of antibodies to the ovarian cancer protein and standard immunoassays. Proteomics and separation techniques may also allow quantification of expression.

In a preferred embodiment, gene expression or protein monitoring of a number of entities, e.g., an expression profile, is monitored simultaneously. Such profiles will typically involve a plurality of those entities described herein.

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In this embodiment, the ovarian cancer nucleic acid probes are attached to biochips as outlined herein for the detection and quantification of ovarian cancer sequences in a narticular cell. Alternatively, PCP may be used. Thus, a series of the continuation of the co

20 particular cell. Alternatively, PCR may be used. Thus, a series, e.g., of microtiter plate, may be used with dispensed primers in desired wells. A PCR reaction can then be performed and analyzed for each well.

Expression monitoring can be performed to identify compounds that modify the expression of one or more ovarian cancer-associated sequences, e.g., a polynucleotide sequence set out inTables 1-26. Generally, in a preferred embodiment, a test modulator is added to the cells prior to analysis. Moreover, screens are also provided to identify agents that modulate ovarian cancer, modulate ovarian cancer proteins, bind to an ovarian cancer protein, or interfere with the binding of an ovarian cancer protein and an antibody or other binding partner.

The term "test compound" or "drug candidate" or "modulator" or grammatical equivalents as used herein describes any molecule, e.g., protein, oligopeptide, small organic molecule, polysaccharide, polynucleotide, etc., to be tested for the capacity to directly or indirectly alter the ovarian cancer phenotype or the expression of an ovarian cancer sequence,

WO 02/102235

PCT/US02/19297

embodiment, the modulator suppresses an ovarian cancer phenotype, e.g., to a normal or nonmalignant tissue fingerprint. In another embodiment, a modulator induced an ovarian cancer concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control, e.g., at zero concentration or below the expression profiles, or expression profile nucleic acids or proteins provided herein. In one phenotype. Generally, a plurality of assay mixtures are run in parallel with different agent e.g., a nucleic acid or protein sequence. In preferred embodiments, modulators alter evel of detection

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than 100 and less than about 2,500 daltons. Preferred small molecules are less than 2000, or groups necessary for structural interaction with proteins, particularly hydrogen bonding, and two of the functional chemical groups. The candidate agents often comprise cyclical carbon organic molecules, preferably small organic compounds having a molecular weight of more or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least more of the above functional groups. Candidate agents are also found among biomolecules ess than 1500 or less than 1000 or less than 500 D. Candidate agents comprise functional Drug candidates encompass numerous chemical classes, though typically they are including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof. Particularly preferred are peptides.

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In one aspect, a modulator will neutralize the effect of an ovarian cancer protein. By "neutralize" is meant that activity of a protein is inhibited or blocked and the consequent

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Conventionally, new chemical entities with useful properties are generated by identifying a e.g., inhibiting activity, creating variants of the lead compound, and evaluating the property and activity of those variant compounds. Often, high throughput screening (HTS) methods chemical compound (called a "lead compound") with some desirable property or activity, In certain embodiments, combinatorial libraries of potential modulators will be screened for an ability to bind to an ovarian cancer polypeptide or to modulate activity. are employed for such an analysis.

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In one preferred embodiment, high throughput screening methods involve providing a compounds). Such "combinatorial chemical libraries" are then screened in one or more assays to identify those library members (particular chemical species or subclasses) that library containing a large number of potential therapeutic compounds (candidate

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conventional "lead compounds" or can themselves be used as potential or actual the rapeutics. display a desired characteristic activity. The compounds thus identified can serve as

can be synthesized through such combinatorial mixing of chemical building blocks. See, e.g., library, such as a polypeptide (e.g., mutein) library, is formed by combining a set of chemical building blocks called amino acids in every possible way for a given compound length (e.g., chemical "building blocks": such as reagents. For example, a linear combinatorial chemical the number of amino acids in a polypeptide compound). Millions of chemical compounds generated by either chemical synthesis or biological synthesis by combining a number of A combinatorial chemical library is a collection of diverse chemical compounds Gallop, et al. (1994) J. Med. Chem. 37:1233-125f. S

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nydantoins, benzodiazepines and dipeptides (Hobbs, et al. (1993) Proc. Nat'l Acad. Sci. USA Preparation and screening of combinatorial chemical libraries is well known to those Publication WO 92/00091), benzodiazepines (U.S. Pat. No. 5,288,514), diversomers such as 91/19735), encoded peptides (PCT Publication WO 93/20242), random bio-oligomers (PCT peptide libraries (see, e.g., U.S. Patent No. 5,010,175; Furka (1991) Pept. Prot. Res. 37:487-493; and Houghton, et al. (1991) Nature 354:84-88), peptoids (PCT Publication No WO of skill in the art. Such combinatorial chemical libraries include, but are not limited to, 90:6909-913), vinylogous polypeptides (Hagihara, et al. (1992) I. Amer. Chem. Soc.

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Med. Chem, 37:1385-401, nucleic acid libraries (see, e.g., Stratagene, Corp.), peptide nucleic Hirschmann, et al. (1992) J. Amer. Chem. Soc. 114:9217-218), analogous organic syntheses Campbell, et al. (1994) J. Org. Chem. 59:658-xxx). See, generally, Gordon, et al. (1994) L. oligocarbamates (Cho, et al. (1993) Science 261:1303-305), and/or peptidyl phosphonates of small compound libraries (Chen, et al. (1994) J. Amer. Chem. Soc. 116:2661-662), 20

114:6568-570), non-peptidal peptidomimetics with a Beta-D-Glucose scaffolding.

al (1996) Nature Biotechnology 14:309-314; and PCT/US96/10287), carbohydrate libraries (see, e.g., Liang, et al. (1996) Science 274:1520-1522; and U.S. Patent No. 5,593,853), and small organic molecule libraries (see, e.g., benzodiazepines, page 33, Baum (Jan. 18, 1993) C&E News; isoprenoids, U.S. Patent No. 5,569,588; thiazolidinones and metathiazanones, acid libraries (see, e.g., U.S. Patent 5,539,083), antibody libraries (see, e.g., Vaughn, et 22

morpholino compounds, U.S. Patent No. 5,506,337; benzodiazepines, U.S. Patent No. U.S. Patent No. 5,549,974; pyrrolidines, U.S. Patent Nos. 5,525,735 and 5,519,134; 5,288,514; and the like). 8

Devices for the preparation of combinatorial libraries are commercially available.

See, e.g., 357 MPS, 390 MPS, Advanced Chem Tech, Louisville KY; Symphony, Rainin, Woburn, MA, 433A Applied Biosystems, Foster City, CA, 9050 Plus, Millipore, Bedford, ₩,

Hewlett-Packard, Palo Alto, CA), which mimic the manual synthetic operations performed by A number of well known robotic systems have also been developed for solution phase apparatus developed by Takeda Chemical Industries, LTD. (Osaka, Japan) and many robotic nature and implementation of modifications to these devices (if any) so that they can operate ComGenex, Princeton, N.J.; Asinex, Moscow, RU; Tripos, Inc., St. Louis, MO; ChemStar, chemistrics. These systems include automated workstations like the automated synthesis a chemist. Any of the above devices are suitable for use with the present invention. The Ltd, Moscow, RU; 3D Pharmaceuticals, Exton, PA; Martek Biosciences, Columbia, MD; systems utilizing robotic arms (Zymate II, Zymark Corporation, Hopkinton, MA; Orca, as discussed herein will be apparent to persons skilled in the relevant art. In addition, numerous combinatorial libraries are themselves commercially available (see, e.g., etc.) 15.

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Preferred assays thus detect enhancement or inhibition of ovarian cancer gene transcription, inhibition or enhancement of polypeptide expression, and inhibition or enhancement of The assays to identify modulators are amenable to high throughput screening. polypeptide activity.

arrays), while U.S. Patent Nos. 5,576,220 and 5,541,061 disclose high throughput methods of Similarly, binding assays and reporter gene assays are similarly well known. Thus, e.g., U.S. No. 5,585,639 discloses high throughput screening methods for nucleic acid binding (e.g., in High throughput assays for the presence, absence, quantification, or other properties Patent No. 5,559,410 discloses high throughput screening methods for proteins, U.S. Patent of particular nucleic acids or protein products are well known to those of skill in the art. screening for ligand/antibody binding. 23 ನ

as a high degree of flexibility and customization. The manufacturers of such systems provide for the assay. These configurable systems provide high throughput and rapid start up as well dispensing, timed incubations, and final readings of the microplate in detector(s) appropriate In addition, high throughput screening systems are commercially available (see, e.g., Instruments, Inc. Fullerton, CA; Precision Systems, Inc., Natick, MA, etc.). These systems typically automate entire procedures, including all sample and reagent pipetting, liquid Zymark Corp., Hopkinton, MA; Air Technical Industries, Mentor, OH; Beckman

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PCT/US02/19297 WO 02/102235

detailed protocols for various high throughput systems. Thus, e.g., Zymark Corp. provides technical bulletins describing screening systems for detecting the modulation of gene transcription, ligand binding, and the like.

useful test compound will be directed to the class of proteins to which the target belongs, e.g., fragments of naturally occurring proteins. Thus, e.g., cellular extracts containing proteins, or preferred in this embodiment are libraries of bacterial, fungal, viral, and mammalian proteins, ibraries of proteins may be made for screening in the methods of the invention. Particularly with the latter being preferred, and human proteins being especially preferred. Particularly In one embodiment, modulators are proteins, often naturally occurring proteins or random or directed digests of proteinaceous cellular extracts, may be used. In this way substrates for enzymes or ligands and receptors. S 2

amino acids, with from about 5 to about 20 amino acids being preferred, and from about 7 to "randomized" or grammatical equivalents herein is meant that each nucleic acid and peptide may incorporate any nucleotide or amino acid at any position. The synthetic process can be these random peptides (or nucleic acids, discussed below) are chemically synthesized, they consists of essentially random nucleotides and amino acids, respectively. Since generally about 15 being particularly preferred. The peptides may be digests of naturally occurring In a preferred embodiment, modulators are peptides of from about 5 to about 30 proteins as is outlined above, random peptides, or "biased" random peptides. By 13

most of the possible combinations over the length of the sequence, thus forming a library of designed to generate randomized proteins or nucleic acids, to allow the formation of all or randomized candidate bioactive proteinaceous agents. ನ

residues are randomized within a defined class, e.g., of hydrophobic amino acids, hydrophilic residues, sterically biased (either small or large) residues, towards the creation of nucleic acid positions within the sequence are either held constant, or are selected from a limited number In one embodiment, the library is fully randomized, with no sequence preferences or constants at any position. In a preferred embodiment, the library is biased. That is, some binding domains, the creation of cysteines, for cross-linking, prolines for SH-3 domains, of possibilities. For example, in a preferred embodiment, the nucleotides or amino acid

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serines, threonines, tyrosines or histidines for phosphorylation sites, etc., or to purines, etc. Modulators of ovarian cancer can also be nucleic acids, as defined above. ဗ္က

As described above generally for proteins, nucleic acid modulating agents may be naturally occurring nucleic acids, random nucleic acids, or "biased" random nucleic acids.

PCT/US02/19297 WO 02/102235

For example, digests of procaryotic or eucaryotic genomes may be used as is outlined above

In a preferred embodiment, the candidate compounds are organic chemical moieties, a wide variety of which are available in the literature.

biochip. If required, the target sequence is prepared using known techniques. For example, with purification and/or amplification such as PCR performed as appropriate. For example, After the candidate agent has been added and the cells allowed to incubate for some the sample may be treated to lyse the cells, using known lysis buffers, electroporation, etc., an in vitro transcription with labels covalently attached to the nucleotides is performed. period of time, the sample containing a target sequence to be analyzed is added to the Generally, the nucleic acids are labeled with biotin-FITC or PE, or with cy3 or cy5.

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or biotin which specifically binds to streptavidin. For the example of biotin, the streptavidin altered by the enzyme. The label also can be a moiety or compound, such as, an epitope tag compound or small molecule, such as an enzyme inhibitor, that binds but is not catalyzed or In a preferred embodiment, the target sequence is labeled with, e.g., a fluorescent, a substrate produces a product that can be detected. Alternatively, the label can be a labeled alkaline phosphatase or horseradish peroxidase, which when provided with an appropriate chemiluminescent, a chemical, or a radioactive signal, to provide a means of detecting the target sequence's specific binding to a probe. The label also can be an enzyme, such as, is labeled as described above, thereby, providing a detectable signal for the bound target sequence. Unbound labeled streptavidin is typically removed prior to analysis.

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5,359,100; 5,124,246; and 5,681,697, each of which is hereby incorporated by reference. In this embodiment, in general, the target nucleic acid is prepared as outlined above, and then assays or can comprise "sandwich assays", which include the use of multiple probes, as is 5,591,584; 5,571,670; 5,580,731; 5,571,670; 5,591,584; 5,624,802; 5,635,352; 5,594,118; As will be appreciated by those in the art, these assays can be direct hybridization added to the biochip comprising a plurality of nucleic acid probes, under conditions that generally outlined in U.S. Patent Nos. 5,681,702; 5,597,909; 5,545,730; 5,594,117; allow the formation of a hybridization complex.

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A variety of hybridization conditions may be used in the present invention, including high, moderate and low stringency conditions as outlined above. The assays are generally run under stringency conditions which allows formation of the label probe hybridization complex only in the presence of target. Stringency can be controlled by altering a step

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PCT/US02/19297 WO 02/102235

formamide concentration, salt concentration, chaotropic salt concentration pH, organic parameter that is a thermodynamic variable, including, but not limited to, temperature, solvent concentration, etc.

outlined in U.S. Patent No. 5,681,697. Thus it may be desirable to perform certain steps at These parameters may also be used to control non-specific binding, as is generally higher stringency conditions to reduce non-specific binding. The reactions outlined herein may be accomplished in a variety of ways. Components assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc., may also be specific or background interactions. Reagents that otherwise improve the efficiency of the other reagents. These include salts, buffers, neutral proteins, e.g., albumin, detergents, etc. used as appropriate, depending on the sample preparation methods and purity of the target. preferred embodiments outlined below. In addition, the reaction may include a variety of which may be used to facilitate optimal hybridization and detection, and/or reduce nonof the reaction may be added simultaneously, or sequentially, in different orders, with 2

expression levels as between states, of individual genes, forming a gene expression profile. The assay data are analyzed to determine the expression levels, and changes in

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embodiment, e.g., for diagnostic applications, having identified differentially expressed genes expression of individual genes. In an another embodiment, screening is performed to identify Screens are performed to identify modulators of the ovarian cancer phenotype. In one particular expression profile, thus preferably generating the associated phenotype. In another screens are performed to identify agents that bind and/or modulate the biological activity of embodiment, screening is performed to identify modulators that can induce or suppress a important in a particular state, screens can be performed to identify modulators that after expressed gene. Again, having identified the importance of a gene in a particular state, modulators that alter a biological function of the expression product of a differentially 22 2

cancer gene expression profile so as to mimic the expression of the gene from normal tissue, In addition screens can be done for genes that are induced in response to a candidate and agent treated ovarian cancer tissue reveals genes that are not expressed in normal tissue modulated in response to the agent. Comparing expression profiles between normal tissue expression pattern leading to a normal expression pattern, or to modulate a single ovarian agent. After identifying a modulator based upon its ability to suppress an ovarian cancer a screen as described above can be performed to identify genes that are specifically

sequences can be identified and used by methods described herein for ovarian cancer genes or identifying agent treated cells. In addition, antibodies can be raised against the agent induced proteins. In particular these sequences and the proteins they encode find use in marking or proteins and used to target novel therapeutics to the treated ovarian cancer tissue sample. or ovarian cancer tissue, but are expressed in agent treated tissue. These agent-specific

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or "contacting" herein is meant that the candidate agent is added to the cells in such a manner Thus, in one embodiment, a test compound is administered to a population of ovarian cancer cells, that have an associated ovarian cancer expression profile. By "administration" accomplished, e.g., PCT US97/01019. Regulatable gene therapy systems can also be used. as to allow the agent to act upon the cell, whether by uptake and intracellular action, or by candidate agent (e.g., a peptide) may be put into a viral construct such as an adenoviral or action at the cell surface. In some embodiments, nucleic acid encoding a proteinaceous retroviral construct, and added to the cell, such that expression of the peptide agent is

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Once the test compound has been administered to the cells, the cells can be washed if period of time. The cells are then harvested and a new gene expression profile is generated, desired and are allowed to incubate under preferably physiological conditions for some

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screens for new drugs that alter the phenotype can be devised. With this approach, the drug target need not be known and need not be represented in the original expression screening Thus, e.g., ovarian cancer or non-malignant tissue may be screened for agents that modulate, e.g., induce or suppress the ovarian cancer phenotype. A change in at least one gene, preferably many, of the expression profile indicates that the agent has an effect on ovarian cancer activity. By defining such a signature for the ovarian cancer phenotype, platform, nor does the level of transcript for the target protein need to change.

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differentially expressed genes are sometimes referred to herein as "ovarian cancer proteins" or a "ovarian cancer modulatory protein". The ovarian cancer modulatory protein may be a fragment, or alternatively, be the full length protein to the fragment encoded by the nucleic acids of the Tables. Preferably, the ovarian cancer modulatory protein is a fragment. In a preferred embodiment, the ovarian cancer amino acid sequence which is used to determine In a preferred embodiment, as outlined above, screens may be done on individual genes and gene products (proteins). That is, having identified a particular differentially expressed gene as important in a particular state, screening of modulators of either the expression of the gene or the gene product itself can be done. The gene products of 2

embodiment, the sequences are naturally occurring allelic variants of a protein encoded by a nucleic acid of the Tables. In another embodiment, the sequences are sequence variants as sequence identity or similarity is encoded by a nucleic acid of the Tables. In another further described herein.

fragment includes a non-transmembrane region. In a preferred embodiment, the fragment has to 24 amino acids long. More preferably the fragment is a soluble fragment. Preferably, the Preferably, the ovarian cancer modulatory protein is a fragment of approximately 14 fragment is kept as a free acid and the N-terminus is a free amine to aid in coupling, e.g., to an N-terminal Cys to aid in solubility. In another embodiment, the C-terminus of the 2

cysteine. Or, the ovarian cancer proteins are conjugated to an immunogenic agent, e.g., to measured by examining parameters described above. A suitable physiological change that effects of the test compounds upon the function of the ovarian cancer polypeptides can be Measurements of ovarian cancer polypeptide activity, or of ovarian cancer or the ovarian cancer phenotype can be performed using a variety of assays. For example, the

affects activity can be used to assess the influence of a test compound on the polypeptides of animals, one can also measure a variety of effects such as, in the case of ovarian cancer associated with tumors, tumor growth, tumor metastasis, neovascularization, hormone this invention. When the functional consequences are determined using intact cells or 15 2

in intracellular second messengers such as cGMP. In the assays of the invention, mammalian northern blots), changes in cell metabolism such as cell growth or pH changes, and changes release, transcriptional changes to both known and uncharacterized genetic markers (e.g., ovarian cancer polypeptide is typically used, e.g., mouse, preferably human.

ovarian cancer polypeptide levels are determined in vitro by measuring the level of protein or For example, an ovarian cancer polypeptide is first contacted with a potential modulator and ELISA and the like with an antibody that selectively binds to the ovarian cancer polypeptide incubated for a suitable amount of time, e.g., from 0.5 to 48 hours. In one embodiment, the Assays to identify compounds with modulating activity can be performed in vitro. mRNA. The level of protein is measured using immunoassays such as western blotting, 23

or a fragment thereof. For measurement of mRNA, amplification, e.g., using PCR, LCR, or letection agents, e.g., fluorescently or radioactively labeled nucleic acids, radioactively or preferred. The level of protein or mRNA is detected using directly or indirectly labeled nybridization assays, e.g., northern hybridization, RNAse protection, dot blotting, are 30

WO 02/102235

enzymatically labeled antibodies, and the like, as described herein.

Alternatively, a reporter gene system can be devised using the ovarian cancer protein with a potential modulator, the amount of reporter gene transcription, translation, or activity CAT, or  $\beta$ -gal. The reporter construct is typically transfected into a cell. After treatment promoter operably linked to a reporter gene such as luciferase, green fluorescent protein, is measured according to standard techniques known to those of skill in the art.

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expressed gene as important in a particular state, screening of modulators of the expression of the gene or the gene product itself can be done. The gene products of differentially expressed protein may be a fragment, or alternatively, be the full length protein to a fragment shown In a preferred embodiment, as outlined above, screens may be done on individual genes are sometimes referred to herein as "ovarian cancer proteins." The ovarian cancer genes and gene products (proteins). That is, having identified a particular differentially

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differentially expressed activity. Moreover, once initial candidate compounds are identified, performed. Typically, the expression of only one or a few genes are evaluated. In another In one embodiment, screening for modulators of expression of specific genes is embodiment, screens are designed to first find compounds that bind to differentially expressed proteins. These compounds are then evaluated for the ability to modulate variants can be further screened to better evaluate structure activity relationships.

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In a preferred embodiment, binding assays are done. In general, purified or isolated nucleic acids are made. For example, antibodies are generated to the protein gene products, gene product is used; that is, the gene products of one or more differentially expressed Alternatively, cells comprising the ovarian cancer proteins can be used in the assays. and standard immunoassays are run to determine the amount of protein present. 20

Thus, in a preferred embodiment, the methods comprise combining an ovarian cancer development of animal models of human disease. In some embodiments, as outlined herein, ovarian cancer protein. Preferred embodiments utilize the human ovarian cancer protein, protein and a candidate compound, and determining the binding of the compound to the although other mammalian proteins, e.g., counterparts, may also be used, e.g., for the variant or derivative ovarian cancer proteins may be used.

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protein or the candidate agent is non-diffusably bound to an insoluble support having isolated sample receiving areas (e.g., a microtiter plate, an array, etc.). The insoluble supports may be Generally, in a preferred embodiment of the methods herein, the ovarian cancer

PCT/US02/19297

suitable insoluble supports include microtiter plates, arrays, membranes and beads. These are made of any composition to which the compositions can be bound, is readily separated from teflon<sup>TM</sup>, etc. Microtiter plates and arrays are especially convenient because a large number surface of such supports may be solid or porous and of any convenient shape. Examples of typically made of glass, plastic (e.g., polystyrene), polysaccharides, nylon or nitrocellulose, of assays can be carried out simultaneously, using small amounts of reagents and samples. soluble material, and is otherwise compatible with the overall method of screening. The

The particular manner of binding of the composition is not crucial so long as it is compatible

with the reagents and overall methods of the invention, maintains the activity of the

binding of the protein or agent, excess unbound material is removed by washing. The sample antibodies (which do not sterically block either the ligand binding site or activation sequence receiving areas may then be blocked through incubation with bovine serum albumin (BSA), chemical crosslinking, the synthesis of the protein or agent on the surface, etc. Following when the protein is bound to the support), direct binding to "sticky" or ionic supports, composition and is non-diffusible. Preferred methods of binding include the use of casein or other innocuous protein or other moiety. 2 13

analogs, etc. Of particular interest are screening assays for agents that have a low toxicity for vitro protein-protein binding assays, electrophoretic mobility shift assays, immunoassays for In a preferred embodiment, the ovarian cancer protein is bound to the support, and a human cells. A wide variety of assays may be used for this purpose, including labeled in antibodies, non-natural binding agents identified in screens of chemical libraries, peptide support and the ovarian cancer protein is added. Novel binding agents include specific test compound is added to the assay. Alternatively, the candidate agent is bound to the protein binding, functional assays (phosphorylation assays, etc.) and the like.

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washing off excess reagent, and determining whether the label is present on the solid support. cancer protein may be done in a number of ways. In a preferred embodiment, the compound cancer protein to a solid support, adding a labeled candidate agent (e.g., a fluorescent label), is labeled, and binding determined directly, e.g., by attaching all or a portion of the ovarian The determination of the binding of the test modulating compound to the ovarian Various blocking and washing steps may be utilized as appropriate. 3

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In some embodiments, only one of the components is labeled, e.g., the proteins (or proteinaceous candidate compounds) can be labeled. Alternatively, more than one

WO 02/102235

PCT/US02/19297

component can be labeled with different labels, e.g., 1251 for the proteins and a fluorophor for the compound. Proximity reagents, e.g., quenching or energy transfer reagents are also

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In one embodiment, the binding of the test compound is determined by competitive binding assay. The competitor is a binding moiety known to bind to the target molecule (e.g., an ovarian cancer protein), such as an antibody, peptide, binding partner, ligand, etc. Under certain circumstances, there may be competitive binding between the compound and the binding moiety, with the binding moiety displacing the compound. In one embodiment, the test compound is labeled. Either the compound, or the competitor, or both, is added first to the protein for a time sufficient to allow binding, if present. Incubations may be performed at a temperature which facilitates optimal activity, typically 4-40° C. Incubation periods are typically optimized, e.g., to facilitate rapid high throughput screening. Typically between 0.1 and 1 hr will be sufficient. Excess reagent is generally removed or washed away. The second component is then added, and the presence or absence of the labeled component is followed, to indicate binding.

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In a preferred embodiment, the competitor is added first, followed by the test compound. Displacement of the competitor is an indication that the test compound is binding to the ovarian cancer protein and thus is capable of binding to, and potentially modulating, the activity of the ovarian cancer protein. In this embodiment, either component can be labeled. Thus, e.g., if the competitor is labeled, the presence of label in the wash solution indicates displacement by the agent. Alternatively, if the test compound is labeled, the presence of the label on the support indicates displacement.

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In an alternative embodiment, the test compound is added first, with incubation and washing, followed by the competitor. The absence of binding by the competitor may indicate that the test compound is bound to the ovarian cancer protein with a higher affinity. Thus, if the test compound is labeled, the presence of the label on the support, coupled with a lack of competitor binding, may indicate.that the test compound is capable of binding to the ovarian cancer protein.

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In a preferred embodiment, the methods comprise differential screening to identity agents that are capable of modulating the activity of the ovarian cancer proteins. In this embodiment, the methods comprise combining an ovarian cancer protein and a competitor in a first sample. A second sample comprises a test compound, an ovarian cancer protein, and a

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competitor. The binding of the competitor is determined for both samples, and a change, or difference in binding between the two samples indicates the presence of an agent capable of binding to the ovarian cancer protein and potentially modulating its activity. That is, if the binding of the competitor is different in the second sample relative to the first sample, the

agent is capable of binding to the ovarian cancer protein.

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Alternatively, differential screening is used to identify drug candidates that bind to the native ovarian cancer protein, but cannot bind to modified ovarian cancer proteins. The structure of the ovarian cancer protein may be modeled, and used in rational drug design to synthesize agents that interact with that site. Drug candidates that affect the activity of an ovarian cancer protein are also identified by screening drugs for the ability to either enhance or reduce the activity of the protein.

Positive controls and negative controls may be used in the assays. Preferably control and test samples are performed in at least triplicate to obtain statistically significant results. Incubation of all samples is for a time sufficient for the binding of the agent to the protein. Following incubation, samples are washed free of non-specifically bound material and the amount of bound, generally labeled agent determined. For example, where a radiolabel is employed, the samples may be counted in a scintillation counter to determine the amount of

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A variety of other reagents may be included in the screening assays. These include reagents like salts, neutral proteins, e.g., albumin, detergents, etc. which may be used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Also reagents that otherwise improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc., may be used. The mixture of components may be added in an order that provides for the requisite binding.

In a preferred embodiment, the invention provides methods for screening for a compound capable of modulating the activity of an ovarian cancer protein. The methods comprise adding a test compound, as defined above, to a cell comprising ovarian cancer proteins. Preferred cell types include almost any cell. The cells contain a recombinant nucleic acid that encodes an ovarian cancer protein. In a preferred embodiment, a library of candidate agents are tested on a plurality of cells.

In one aspect, the assays are evaluated in the presence or absence or previous or subsequent exposure of physiological signals, e.g., hormones, antibodies, peptides, antigens, cytokines, growth factors, action potentials, pharmacological agents including

chemotherapeutics, radiation, carcinogenics, or other cells (e.g., cell-cell contacts). In another example, the determinations are determined at different stages of the cell cycle process.

In this way, compounds that modulate ovarian cancer agents are identified.

Compounds with pharmacological activity are able to enhance or interfere with the activity of the ovarian cancer protein. Once identified, similar structures are evaluated to identify critical structural feature of the compound. In one embodiment, a method of inhibiting ovarian cancer cell division is provided.

The method comprises administration of an ovarian cancer inhibitor. In another embodiment, an ovarian cancer inhibitor. In a further embodiment, methods of treating cells or individuals with ovarian cancer are provided. The method comprises administration of an ovarian cancer a method of inhibiting ovarian cancer is provided. The method comprises administration of

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In one embodiment, an ovarian cancer inhibitor is an antibody as discussed above. In another embodiment, the ovarian cancer inhibitor is an antisense or RNAi molecule.

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A variety of cell viability, growth, proliferation, and metastasis assays are known to those of skill in the art, as described below.

Soft agar growth or colony formation in suspension

abnormal cellular proliferation and transformation. A therapeutic compound would reduce or transformed, they lose this phenotype and grow detached from the substrate. For example, transformed cells can grow in stirred suspension culture or suspended in semi-solid media, eliminate the host cells' ability to grow in stirred suspension culture or suspended in semisuppressor genes, regenerate normal phenotype and require a solid substrate to attach and grow. Soft agar growth or colony formation in suspension assays can be used to identify Normal cells require a solid substrate to attach and grow. When the cells are such as semi-solid or soft agar. The transformed cells, when transfected with tumor modulators of ovarian cancer sequences, which when expressed in host cells, inhibit solid media, such as semi-solid or soft. 25 ន

Wiley-Liss, herein incorporated by reference. See also, the methods section of Garkaytsev, et described in Freshney (1994) Culture of Animal Cells: A Manual of Basic Technique (3d ed.) Techniques for soft agar growth or colony formation in suspension assays are al. (1996), supra, herein incorporated by reference.

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Contact inhibition and density limitation of growth

continue to grow to high densities in disorganized foci. Thus, the transformed cells grow to a Normal cells typically grow in a flat and organized pattern in a petri dish until they pattern of normal surrounding cells. Alternatively, labeling index with (3H)-thymidine at higher saturation density than normal cells. This can be detected morphologically by the formation of a disoriented monolayer of cells or rounded cells in foci within the regular touch other cells. When the cells touch one another, they are contact inhibited and stop growing. When cells are transformed, however, the cells are not contact inhibited and S

saturation density can be used to measure density limitation of growth. See, e.g., Freshney regenerate a normal phenotype and become contact inhibited and would grow to a lower (1994), supra. The transformed cells, when transfected with tumor suppressor genes, 12

with an ovarian cancer-associated sequence and are grown for 24 hr at saturation density in In this assay, labeling index with (3H)-thymidine at saturation density is a preferred method of measuring density limitation of growth. Transformed host cells are transfected non-limiting medium conditions. The percentage of cells labeling with (<sup>3</sup>H)-thymidine is determined autoradiographically. See, e.g., Freshney (1994), supra.

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### Growth factor or serum dependence 2

Exp. Med. 131:836-879; and Freshney, supra. This is in part due to release of various growth counterparts. See, e.g., Temin (1966) J. Nat'l Cancer Inst., 37:167-175; Eagle, et al. (1970) J. factors by the transformed cells. Growth factor or serum dependence of transformed host Transformed cells typically have a lower serum dependence than their normal

cells can be compared with that of control. 22

### Tumor specific markers levels

specific markers") than their normal counterparts. For example, plasminogen activator (PA) Gullino, pp. 178-184 "Angiogenesis, tumor vascularization, and potential interference with tumor growth" in Mihich (ed. 1985) Biological Responses in Cancer Plenum. Similarly, is released from human glioma at a higher level than from normal brain cells (see, e.g., Tumor cells release an increased amount of certain factors (hereinafter "tumor

tumor angiogenesis factor (TAF) is released at a higher level in tumor cells than their normal counterparts. Sec, c.g., Folkman (1992) Sem Cancer Biol, 3:89-96.

Strickland and Beers (1976) J. Biol. Chem. 251:5694-5702; Whur, et al. (1980) Br. J. Cancer Freshney (1994), supra. Also, see, Unkeless, et al. (1974) J. Biol. Chem. 249:4295-4305; 42:305-312; Gullino, pp. 178-184 "Angiogenesis, tumor vascularization, and potential Various techniques which measure the release of these factors are described in interference with tumor growth" in Mihich (ed. 1985) Biological Responses in Cancer Plenum; and Freshney (1985) Anticancer Res, 5:111-130.

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### Invasiveness into Matrigel 10

assay, tumorigenic cells are typically used as host cells. Expression of a tumor suppressor invasiveness of cells into Matrigel or some other extracellular matrix constituent. In this constituent can be used as an assay to identify compounds that modulate ovarian cancerassociated sequences. Tumor cells exhibit a good correlation between malignancy and The degree of invasiveness into Matrigel or some other extracellular matrix gene in these host cells would decrease invasiveness of the host cells.

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coated with Matrigel or some other extracellular matrix constituent. Penetration into the gel, or through to the distal side of the filter, is rated as invasiveness, and rated histologically by number of cells and distance moved, or by pre-labeling the cells with 125I and counting the radioactivity on the distal side of the filter or bottom of the dish. See, e.g., Freshney (1984), Alternatively, the level of invasion of host cells can be measured by using filters supra.

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### Tumor growth in vivo

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transgenic or immune-suppressed mice. Knock-out transgenic mice can be made, in which the ovarian cancer gene is disrupted or in which an ovarian cancer gene is inserted. Knockrecombination. Such mice can also be made by substituting the endogenous ovarian cancer out transgenic mice can be made by insertion of a marker gene or other heterologous gene gene with a mutated version of the ovarian cancer gene, or by mutating the endogenous Effects of ovarian cancer-associated sequences on cell growth can be tested in into the endogenous ovarian cancer gene site in the mouse genome via homologous ovarian cancer gene, e.g., by exposure to carcinogens.

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WO 02/102235

PCT/US02/19297

containing the newly engineered genetic lesion are injected into a host mouse embryo, which mice it is possible to obtain a new line of mice containing the introduced genetic lesion. See, hat possess germ cells partially derived from the mutant cell line. By breeding the chimeric e.g., Capecchi, et al. (1989) Science 244:1288-1292. Chimeric targeted mice can be derived is re-implanted into a recipient female. Some of these embryos develop into chimeric mice according to Hogan, et al. (1988) Manipulating the Mouse Embryo: A Laboratory Manual CSH Press; and Robertson (ed. 1987) Teratocarcinomas and Embryonic Stem Cells: A A DNA construct is introduced into the nuclei of embryonic stem cells. Cells Practical Approach IRL Press, Washington, D.C.

cells) injected into isogenic hosts will produce invasive tumors in a high proportions of cases, while normal cells of similar origin will not. In hosts which developed invasive tumors, cells used. For example, genetically athymic "nude" mouse (see, e.g., Giovanella, et al. (1974) L. suitable length of time, preferably 4-8 weeks, tumor growth is measured (e.g., by volume or Alternatively, various immune-suppressed or immune-deficient host animals can be mouse (see, e.g., Bradley, et al. (1978) Br. J. Cancer 38:263-272; Selby, et al. (1980) Br. J. by its two largest dimensions) and compared to the control. Tumors that have statistically Cancer 41:52-61) can be used as a host. Transplantable tumor cells (typically about 106 Nat! Cancer Inst. 52:921-930), a SCID mouse, a thymectomized mouse, or an irradiated expressing an ovarian cancer-associated sequences are injected subcutaneously. After a 2 2

## Polynucleotide modulators of ovarian cancer

significant reduction (using, e.g., Student's T test) are said to have inhibited growth.

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Antisense and RNAi Polynucleotides

Binding of the antisense polynucleotide to the mRNA reduces the translation and/or stability In certain embodiments, the activity of an ovarian cancer-associated protein is downregulated, or entirely inhibited, by the use of antisense polynucleotide, e.g., a nucleic acid nucleic acid sequence, e.g., an ovarian cancer protein mRNA, or a subsequence thereof. complementary to, and which can preferably hybridize specifically to, a coding mRNA of the mRNA. 25

occurring nucleotides, or synthetic species formed from naturally-occurring subunits or their close homologs. Antisense polynucleotides may also have altered sugar moieties or inter-In the context of this invention, antisense polynucleotides can comprise naturally-30

sugar linkages. Exemplary among these are the phosphorothioate and other sulfur containing species which are known for use in the art. Analogs are comprehended by this invention so long as they function effectively to hybridize with the ovarian cancer protein mRNA. See, e.g., Isis Pharmaceuticals, Carlsbad, CA; Sequitor, Inc., Natick, MA.

Such antisense polynucleotides can readily be synthesized using recombinant means, or can be synthesized in vitro. Equipment for such synthesis is sold by several vendors, including Applied Biosystems. The preparation of other oligonucleotides such as phosphorothioates and alkylated derivatives is also well known to those of skill in the art.

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Antisense molecules as used herein include antisense or sense oligonucleotides. Sense oligonucleotides can, e.g., be employed to block transcription by binding to the antisense strand. The antisense and sense oligonucleotide comprise a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target mRNA (sense) or DNA (antisense) sequences for ovarian cancer molecules. A preferred antisense molecule is for an ovarian cancer sequences in Tables 1-26, or for a ligand or activator thereof. Antisense or sense oligonucleotides, according to the present invention, comprise a fragment generally at least about 14 nucleotides, preferably from about 14 to 30 nucleotides. An antisense or a sense oligonucleotide can be developed based upon a cDNA sequence encoding a given protein. See, e.g., Stein and Cohen (1988) Cancer Res, 48:2659-2668; and van der Krol, et al. (1988) <u>BioTechniques</u> 6:958-976.

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manner. See, e.g., Brumelkamp, et al. (2002) <u>Sciencexpress</u> (21March2002); Sharp (1999) <u>Genes Dev.</u> 13:139-141; and Cathew (2001) <u>Curr. Op. Cell Biol.</u> 13:244-248. In mammalian cells, short, e.g., 21 nt, double stranded small interfering RNAs (siRNA) have been shown to be effective at inducing an RNAi response. See, e.g., Elbashir, et al. (2001) <u>Nature</u> 411:494-25. 498. The mechanism may be used to down-regulate expression levels of identified genes, e.g., treatment of or validation of relevance to disease.

#### Ribozymes

In addition to antisense polynucleotides, ribozymes can be used to target and inhibit transcription of ovarian cancer-associated nucleotide sequences. A ribozyme is an RNA molecule that catalytically cleaves other RNA molecules. Different kinds of ribozymes have been described, including group I ribozymes, hammerhead ribozymes, hairpin ribozymes, RNase P, and axhead ribozymes (see, e.g., Castanotto, et al. (1994) Adv. Pharmacol. 25: 289-

WO 02/102235 PCT/US02/19297

317 for a general review of the properties of different ribozymes).

The general features of hairpin ribozymes are described, e.g., in Hampel, et ai. (1990) Nucl. Acids Res. 18:299-304; Buropean Patent Publication No. 0 360 257; U.S. Patent No. 5,254,678. Methods of preparing them are well known to those of skill in the art. See, e.g., WO 94/26877; Ojwang, et al. (1993) Proc. Nat'l Acad. Sci. USA 90:6340-6344; Yamada, et al. (1994) Hum. Gene Ther. 1:39-45; Leavitt, et al. (1995) Proc. Nat'l Acad. Sci. USA 92:699-703; Leavitt, et al. (1994) Hum. Gene Ther. 5:1151-120; and Yamada, et al. (1994)

Polynucleotide modulators of ovarian cancer may be introduced into a cell containing the target nucleotide sequence by formation of a conjugate with a ligand binding molecule, as described in WO 91/04753. Suitable ligand binding molecules include, but are not limited to, cell surface receptors, growth factors, other cytokines, or other ligands that bind to cell surface receptors. Preferably, conjugation of the ligand binding molecule does not substantially interfere with the ability of the ligand binding molecule to bind to its corresponding molecule or receptor, or block entry of the sense or antisense oligonucleotide or its conjugated version into the cell. Alternatively, a polynucleotide modulator of ovarian cancer may be introduced into a cell containing the target nucleic acid sequence, e.g., by formation of an polynucleotide-lipid complex, as described in WO 90/10448. It is understood that the use of antisense molecules or knock out and knock in models may also be

formation of an polynucleotide-lipid complex, as described in WO 90/10448. It is understood that the use of antisense molecules or knock out and knock in models may also be used in screening assays as discussed above, in addition to methods of treatment.

Thus, in one embodiment, methods of modulating ovarian cancer in cells or organisms are provided. In one embodiment, the methods comprise administering to a cell an anti-ovarian cancer antibody that reduces or eliminates the biological activity of an

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endogenous ovarian cancer protein. Alternatively, the methods comprise administering to a cell or organism a recombinant nucleic acid encoding an ovarian cancer protein. This may be accomplished in any number of ways. In a preferred embodiment, e.g., when the ovarian cancer sequence is down-regulated in ovarian cancer, such state may be reversed by increasing the amount of ovarian cancer gene product in the cell. This can be accomplished, e.g., by over-expressing the endogenous ovarian cancer gene or administering a gene encoding the ovarian cancer sequence, using known gene-therapy techniques, e.g.. In a

encoding the ovarian cancer sequence, using known gene-therapy techniques, e.g.. In a preferred embodiment, the gene therapy techniques include the incorporation of the exogenous gene using enhanced homologous recombination (EHR), e.g., as described in PCT/US93/03868, hereby incorporated by reference in its entirety. Alternatively, e.g., when

WO 02/10223S

PCT/US02/19297

the ovarian cancer sequence is up-regulated in ovarian cancer, the activity of the endogenous ovarian cancer gene is decreased, e.g., by the administration of an ovarian cancer antisense or RNAi nucleic acid.

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In one embodiment, the ovarian cancer proteins of the present invention may be used to generate polyclonal and monoclonal antibodies to ovarian cancer proteins. Similarly, the ovarian cancer proteins can be coupled, using standard technology, to affinity chromatography columns. These columns may then be used to purify ovarian cancer antibodies useful for production, diagnostic, or therapeutic purposes. In a preferred embodiment, the antibodies are generated to epitopes unique to an ovarian cancer protein; that is, the antibodies show little or no cross-reactivity to other proteins. The ovarian cancer antibodies may be coupled to standard affinity chromatography columns and used to purify ovarian cancer proteins. The antibodies may also be used as blocking polypeptides, as outlined above, since they will specifically bind to the ovarian cancer protein.

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# 15 Methods of identifying variant ovarian cancer-associated sequences

Without being bound by theory, expression of various ovarian cancer sequences is correlated with ovarian cancer. Accordingly, disorders based on mutant or variant ovarian cancer genes may be determined. In one embodiment, the invention provides methods for identifying cells containing variant ovarian cancer genes, e.g., determining all or part of the sequence of at least one endogenous ovarian cancer genes in a cell. This may be accomplished using any number of sequencing techniques. In a preferred embodiment, the invention provides methods of identifying the ovarian cancer gene of an individual, e.g., determining all or part of the sequence of at least one ovarian cancer gene of the individual. This is generally done in at least one tissue of the individual, and may include the evaluation of a number of tissues or different samples of the same tissue. The method may include comparing the sequence of the sequenced ovarian cancer gene to a known ovarian cancer gene, a wild-type gene.

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The sequence of all or part of the ovarian cancer gene can then be compared to the sequence of a known ovarian cancer gene to determine if any differences exist. This can be done using any number of known homology programs, such as Bestfit, etc. In a preferred embodiment, the presence of a difference in the sequence between the ovarian cancer gene of the patient and the known ovarian cancer gene correlates with a disease state or a propensity for a disease state, as outlined herein.

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In a preferred embodiment, the ovarian cancer genes are used as probes to determine the number of copies of the ovarian cancer gene in the genome.

In another preferred embodiment, the ovarian cancer genes are used as probes to determine the chromosomal localization of the ovarian cancer genes. Information such as chromosomal localization finds use in providing a diagnosis or prognosis in particular when chromosomal abnormalities such as translocations, and the like are identified in the ovarian cancer gene locus.

# Administration of pharmaceutical and vaccine compositions

10 In one embodiment, a therapeutically effective dose of an ovarian cancer protein or modulator thereof, is administered to a patient. By "therapeutically effective dose" herein is meant a dose that produces effects for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques. See, e.g., Ansel, et al. (1999) <u>Pharmaceutical Dosage Forms and Drug</u>
15 <u>Delivery Systems Lippincott</u>; <u>Lieberman (1992) <u>Pharmaceutical Dosage Forms and Drug</u>
25 <u>Dekker</u>, ISBN 0824770846, 082476918X, 0824712692, 0824716981; <u>Lloyd (1999) <u>The Art.</u>
Science and <u>Technology of Pharmaceutical Compounding Amer.</u> Pharmaceutical Assn.; and Pickar (1999) <u>Dosage Calculations</u> Thomson. Adjustments for ovarian cancer degradation, systemic versus localized delivery, and rate of new protease synthesis, as well as the age,</u></u>

body weight, general health, sex, diet, time of administration, drug interaction, and the severity of the condition may be necessary, and will be ascertainable with routine experimentation by those skilled in the art. U.S. Patent Application No. 09/687,576, further discloses the use of compositions and methods of diagnosis and treatment in ovarian cancer is hereby expressly incorporated by reference.

animals, particularly mammals. Thus the methods are applicable to both humans and other veterinary applications. In the preferred embodiment the patient is a mammal, preferably a primate, and in the most preferred embodiment the patient is human.

The administration of the ovarian cancer proteins and modulators thereof of the present invention can be done in a variety of ways as discussed above, including, but not limited to, orally, subcutaneously, intravenously, intra-nasally, transdermally, intraperitoneally, intramuscularly, intrapulmonary, vaginally, rectally, or intraocularly. In some instances, e.g., in the treatment of wounds and inflammation, the ovarian cancer

WO 02/102235

PCT/US02/19297

proteins and modulators may be directly applied as a solution or spray.

The pharmaceutical compositions of the present invention comprise an ovarian cancer "Pharmaceutically acceptable base addition salts" include those derived from inorganic bases methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like. substituted amines including naturally occurring substituted amines, cyclic amines and basic sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic protein in a form suitable for administration to a patient. In the preferred embodiment, the ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, such as sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, salts. "Pharmaceutically acceptable acid addition salt" refers to those salts that retain the pharmaceutically acceptable salts, which is meant to include both acid and base addition potassium, sodium, calcium, and magnesium salts. Salts derived from pharmaceutically acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, undesirable, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, biological effectiveness of the free bases and that are not biologically or otherwise manganese, aluminum salts and the like. Particularly preferred are the ammonium, pharmaceutical compositions are in a water soluble form, such as being present as tripropylamine, and ethanolamine.

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The pharmaceutical compositions may also include one or more of the following: carrier proteins such as serum albumin; buffers, fillers such as microcrystalline cellulose, lactose, corn and other starches; binding agents; sweeteners and other flavoring agents; coloring agents; and polyethylene glycol.

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forms depending upon the method of administration. For example, unit dosage forms suitable for oral administration include, but are not limited to, powder, tablets, pills, capsules, and lozenges. It is recognized that ovarian cancer protein modulators (e.g., antibodies, antisense constructs, ribozymes, small organic molecules, etc.) when administered orally, should be protected from digestion. This is typically accomplished either by complexing the molecule(s) with a composition to render it resistant to acidic and enzymatic hydrolysis, or by packaging the molecule(s) in an appropriately resistant carrier, such as a liposome or a protection barrier. Means of protecting agents from digestion are well known in the art.

The compositions for administration will commonly comprise an ovarian cancer protein modulator dissolved in a pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., buffered saline and the like. These solutions are sterile and generally free of undesirable matter. These compositions may be

about 100 mg per patient per day may be used, particularly when the drug is administered to a odium lactate and the like. The concentration of active agent in these formulations can vary physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents secluded site and not into the blood stream, such as into a body cavity or into a lumen of an widely, and will be selected primarily based on fluid volumes, viscosities, body weight, and organ. Substantially higher dosages are possible in topical administration. Actual methods the like in accordance with the particular mode of administration selected and the patient's needs. See, e.g., Remington's Pharmaceutical Science (15th ed., 1980) and Hardman and and the like, e.g., sodium acetate, sodium chloride, potassium chloride, calcium chloride, Limbird (eds. 2001) Goodman and Gillman: The Pharmacological Basis of Therapeutics administration would be about 0.1 to 10 mg per patient per day. Dosages from 0.1 up to sterilized by conventional, well known sterilization techniques. The compositions may (10th ed.) McGraw-Hill. Thus, a typical pharmaceutical composition for intravenous contain pharmaceutically acceptable auxiliary substances as required to approximate for preparing parenterally administrable compositions are readily available. S 2 15

The compositions containing modulators of ovarian cancer proteins can be administered for therapeutic or prophylactic treatments. In therapeutic applications, compositions are administered to a patient suffering from a disease (e.g., a cancer) in an amount sufficient to cure or at least partially arrest the disease and/or its complications. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for this use will depend upon the severity of the disease and the general state of the patient's health. Single or multiple administrations of the compositions may be administered depending on the dosage and frequency as required and tolerated by the patient. In any event, the composition should provide a sufficient quantity of the agents of this invention to effectively treat the patient. An amount of modulator that is capable of preventing or slowing the development of cancer in a mammal is referred to as a "prophylactically effective dose."

The particular dose required for a prophylactic treatment will depend upon the medical

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factors such as age, weight, gender, administration route, efficiency, etc. Such prophylactic

condition and history of the mammal, the particular cancer being prevented, as well as other

recurrence of the cancer, or in a mammal who is suspected of having a significant likelihood of developing cancer based, e.g., in part, upon gene expression profiles. Vaccine strategies treatments may be used, e.g., in a mammal who has previously had cancer to prevent a may be used, in either a DNA vaccine form, or protein vaccine. It will be appreciated that the present ovarian cancer protein-modulating compounds can be administered alone or in combination with additional ovarian cancer modulating compounds or with other therapeutic agent, e.g., other anti-cancer agents or treatments.

cancer-associated polypeptides and nucleic acids using in vitro (cell-free), ex vivo or in vivo polynucleotides or ribozymes, will be introduced into cells, in vitro or in vivo. The present invention provides methods, reagents, vectors, and cells useful for expression of ovarian comprising nucleic acid sequences set forth in Tables 1-26, such as RNAi, antisense In numerous embodiments, one or more nucleic acids, e.g., polynucleotides (cell or organism-based) recombinant expression systems.

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plasma vectors, viral vectors and any of the other well known methods for introducing cloned genomic DNA, cDNA, synthetic DNA or other foreign genetic material into a host cell. See, Enzymology (vol. 152) Academic Press, Ausubel, et al. (eds. 1999 and supplements) Current introducing foreign nucleotide sequences into host cells may be used. These include the use of calcium phosphate transfection, spheroplasts, electroporation, liposomes, microinjection, Protocols Lippincott; and Sambrook, et al. (2001) Molecular Cloning: A Laboratory Manual e.g., Berger and Kimmel (1987) Guide to Molecular Cloning Techniques from Methods in The particular procedure used to introduce the nucleic acids into a host cell for expression of a protein or nucleic acid is application specific. Many procedures for (3d ed., Vol. 1-3) CSH Press.

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genes (including both the full-length sequence, partial sequences, or regulatory sequences of the ovarian cancer coding regions) can be administered in a gene therapy application. These In a preferred embodiment, ovarian cancer proteins and modulators are administered incorporation into the genome) or as antisense compositions, as will be appreciated by those as therapeutic agents, and can be formulated as outlined above. Similarly, ovarian cancer ovarian cancer genes can include antisense applications, either as gene therapy (e.g., for

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Ovarian cancer polypeptides and polynucleotides can also be administered as vaccine compositions to stimulate HTL, CII,, and antibody responses.. Such vaccine compositions can include, e.g., lipidated peptides (see, e.g., Vitiello, et al. (1995) J. Clin, Invest. 95:341-

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Cytokines, such as GM-CSF, interleukin-2, -7, -12, and other like growth factors, may also be

polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A.

prosine; acylated sugars; cationically or anionically derivatized polysaccharides;

PCT/US02/19297 WO 02/102235

(1994) Vaccine 12:299-306; Jones, et al. (1995) Vaccine 13:675-681), peptide compositions microspheres (see, e.g., Eldridge, et al. (1991) Molec. Immunol. 28:287-294; Alonso, et al. contained in immune stimulating complexes (ISCOMS; see, e.g., Takahashi, et al. (1990) 349), peptide compositions encapsulated in poly(D,L-lactide-co-glycolide, "PLG")

- vectors (Perkus, et al., p. 379, in Kaufmann (ed. 1996) Concepts in Vaccine Development de Nature 344:873-875; Hu, et al. (1998) Clin. Exp. Immunol. 113:235-243), multiple antigen Tam (1996) J. Immunol. Methods 196:17-32), peptides formulated as multivalent peptides, peptide systems (MAPs; see, e.g., Tam (1988) Proc. Nat'l Acad. Sci. USA 85:5409-5413; peptides for use in ballistic delivery systems, typically crystallized peptides, viral delivery
- origin (see, e.g., Koffer, et al. (1996) <u>I. Immunol. Methods</u> 192:25-35; Bldridge, et al. (1993) Sem. Hematol. 30:16-24; Falo, et al. (1995) Nature Med. 7:649-653), adjuvants (Warren, et 540; Kieny, et al. (1986) <u>AIDS Bio/Technology</u> 4:790-795; Top, et al. (1971) <u>1. Infect. Dis.</u> 7:131-137), or, naked or particle absorbed cDNA (Ulmer, et al. (1993) Science 259:1745liposomes (Reddy, et al. (1992) J. Immunol, 148:1585-1589; Rock (1996) Immunol, Today Gruyter; Chakrabarti, et al. (1986) Nature 320:535-537; Hu, et al. (1986) Nature 320:537-(24:148-154; Chanda, et al. (1990) <u>Virology</u> 175:535-547), particles of viral or synthetic al. (1986) Ann. Rev. Immunol, 4:369-388; Gupta, et al. (1993) Vaccine 11:293-306), 2 15
- 996) Concepts in Vaccine Development de Gruyter; Cease and Berzofsky (1994) Ann. Rev. Immunol. 12:923-989; and Eldridge, et al. (1993) Sem. Hematol. 30:16-24). Toxin-targeted delivery technologies, also known as receptor mediated targeting, such as those of Avant immunotherapeutics, Inc. (Needham, Massachusetts) may also be used. ຊ

.749; Robinson, et al. (1993) <u>Vaccing</u> 11:957-960; Shiver, et al., p. 423, in Kaufmann (ed.

Mycobacterium tuberculosis derived proteins. Certain adjuvants are commercially available as, e.g., Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, Vaccine compositions often include adjuvants. Many adjuvants contain a substance mineral oil, and a stimulator of immune responses, such as lipid A, Bortadella pertussis, or Beecham, Philadelphia, PA); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NI); AS-2 (SmithKline 25 2

used as adjuvants.

See, e.g., Wolff et. al. (1990) Science 247:1465-1468; U.S. Patent Nos. 5,580,859, 5,589,466; mediated) delivery, cationic lipid complexes, and particle-mediated ("gene gun") or pressureencoding one or more of the polypeptides, or a fragment thereof, is administered to a patient. 5,804,566; 5,739,118; 5,736,524; 5,679,647; and WO 98/04720. Examples of DNA-based Vaccines can be administered as nucleic acid compositions wherein DNA of RNA delivery technologies include "naked DNA", facilitated (bupivicaine, polymers, peptidemediated delivery (see, e.g., U.S. Patent No. 5,922,687). For therapeutic or prophylactic immunization purposes, the peptides of the invention vaccinia virus expresses the immunogenic peptide, and thereby elicits an immune response. Vaccinia vectors and methods useful in immunization protocols are described in, e.g., U.S. vaccinia virus, e.g., as a vector to express nucleotide sequences that encode ovarian cancer polypeptides or polypeptide fragments. Upon introduction into a host, the recombinant can be expressed by viral or bacterial vectors. Examples of expression vectors include attenuated viral hosts, such as vaccinia or fowlpox. This approach involves the use of 2 2

described in Stover, et al. (1991) Nature 351:456-460. A wide variety of other vectors useful vectors, retroviral vectors, Salmonella typhi vectors, detoxified anthrax toxin vectors, and the like, will be apparent. See, e.g., Shata, et al. (2000) Mol. Med. Today 6:66-71; Shedlock, et Patent No. 4,722,848. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are for therapeutic administration or immunization e.g., adeno and adeno-associated virus al. (2000) J. Leukoc. Biol. 68:793-806; and Hipp, et al. (2000) In Vivo 14:571-85. 2

gene. For example, ovarian cancer-associated genes or sequence encoding subfragments of an responses. This procedure provides for production of cytotoxic T cell responses against cells an ovarian cancer gene or portion of an ovarian cancer gene under the control of a regulatable DNA vaccine comprising a plurality of nucleotide sequences derived from an ovarian cancer derived from the ovarian cancer protein. In one embodiment, a patient is immunized with a Methods for the use of genes as DNA vaccines are well known, and include placing ovarian cancer gene used for DNA vaccines can encode full-length ovarian cancer proteins, immunogenicity in the context of Class I MHC and an ability to generate cytotoxic T cell promoter or a tissue-specific promoter for expression in an ovarian cancer patient. The but more preferably encodes portions of the ovarian cancer proteins including peptides ovarian cancer protein are introduced into expression vectors and tested for their which present antigen, including intracellular epitopes.

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PCT/US02/19297 WO 02/102235

the immunogenic response to the ovarian cancer polypeptide encoded by the DNA vaccine. In a preferred embodiment, the DNA vaccines include a gene encoding an adjuvant molecule with the DNA vaccine. Such adjuvant molecules include cytokines that increase Additional or alternative adjuvants are available.

- In another preferred embodiment ovarian cancer genes find use in generating animal diminished in cancer tissue, gene therapy technology, e.g., wherein antisense RNA directed to the ovarian cancer gene will also diminish or repress expression of the gene. Animal models of ovarian cancer find use in screening for modulators of an ovarian cancermodels of ovarian cancer. When the ovarian cancer gene identified is repressed or
- increased expression of the ovarian cancer protein. When desired, tissue-specific expression recombination with an appropriate gene targeting vector, will result in the absence or associated sequence or modulators of ovarian cancer. Similarly, transgenic animal technology including gene knockout technology, e.g., as a result of homologous or knockout of the ovarian cancer protein may be necessary. 2
- Depending on the desired expression level, promoters of various strengths can be employed It is also possible that the ovarian cancer protein is overexpressed in ovarian cancer. As such, transgenic animals can be generated that overexpress the ovarian cancer protein. to express the transgene. Also, the number of copies of the integrated transgene can be determined and compared for a determination of the expression level of the transgene. 2
- Animals generated by such methods find use as animal models of ovarian cancer and are dditionally useful in screening for modulators to treat ovarian cancer. 2

# Kits for Use in Diagnostic and/or Prognostic Applications

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For use in diagnostic, research, and therapeutic applications suggested above, kits are acids or antibodies, hybridization probes and/or primers, siRNA or antisense polynucleotides, molecules inhibitors of ovarian cancer-associated sequences etc. A therapeutic product may include sterile saline or another pharmaceutically acceptable emulsion and suspension base. include any or all of the following: assay reagents, buffers, ovarian cancer-specific nucleic also provided by the invention. In the diagnostic and research applications such kits may ibozymes, dominant negative ovarian cancer polypeptides or polynucleotides, small 30

protocols) for the practice of the methods of this invention. While the instructional materials In addition, the kits may include instructional materials containing directions (e.g., typically comprise written or printed materials they are not limited to such. Any medium

WO 02/102235

PCT/US02/19297

capable of storing such instructions and communicating them to an end user is contemplated by this invention. Such media include, but are not limited to electronic storage media (e.g., media may include addresses to internet sites that provide such instructional materials. magnetic discs, tapes, cartridges, chips), optical media (e.g., CD ROM), and the like.

the present invention, depending upon the intended user of the kit and the particular needs of cancer-associated sequences. Such kits can be prepared from readily available materials and ovarian cancer protein. A wide variety of kits and components can be prepared according to ovarian cancer-associated polypeptide or polynucleotide, reaction tubes, and instructions for The genes will be selected based on correlations with important parameters in disease which The present invention also provides for kits for screening for modulators of ovarian the user. Diagnosis would typically involve evaluation of a plurality of genes or products. reagents. For example, such kits can comprise one or more of the following materials: an testing ovarian cancer-associated activity. Optionally, the kit contains biologically active may be identified in historical or outcome data.

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#### EXAMPLES

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## Example 1: Gene Chip Analysis

described (Glynne, et al. (2000) Nature 403:672-676; Zhao, et al. (2000) Genes Dev. 14:981-Molecular profiles of various normal and cancerous tissues were determined and analyzed using gene chips. RNA was isolated and gene chip analysis was performed as 933) 2

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BE313280 241698 NM\_014781 AF035528 A086060 D25983 BE342701 NM\_006159 A1660247

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cagulation factor XIII. A1 polypeptide

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TABLE 21C.

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TABLE 22A:

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eminderulinate, della-, dehyd	hypothetical protein FLJ10512	NM_007116":	ESTs	DKFZP5851_2024 protein	EGF-containing Spuilty-like ex	ESTs	hypothetical protein DKFZp586H	EST	EST	ESTa	ESTs	MAADA38 pene groduct	ESTs	KIAAG878 protein	integrin, stohe 8	EST	Pypotholical protein PLJ23191	ESTs	Human DNA seguence from clone	UCC1 proteth	hypothetical protein FLJ21939	myopodin	cavedin 2	gb:L3,C70214-161299-045-806 C	EST	ESTs	Homo saplens cONA: FLJ22042 fi	carboxypeplidase Z	6.2 ld protein	Homo saplens mRNA; cDNA DKF2p4	ESTs	ESTs	ESTs	
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AA151529	AA767718		A443966	AL11764	AA301867	AA335769	AA017472	AP209158	194907	AK84710	A493206	ALCOR924	AI6S4048	NM_014899	136331	AI695975	AI6S4223	BE326214	T03667	BE016682	AA732038	AW295374	AW358578	AW752597	N50432	W73853	AW119112	NM_003652	AW957295	AL133047	AA232635	AB71055	141160	
444815	420728	404245	436420	410058	414478	424137	47859	444862	426088	436080	424651	432939	449088	428842	419577	450435	450698	421255	432467	408654	412611	453355	424865	458147	447568	414496	425618	415166	422157	450253	418919	444846	418781	
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	408458	106033_1	AUDDITA ALDSTSZ BEDOOGB AAJTGITB IITZEG AAJASSG AAJASSG AASARGOOS RTGISG RTIOGG AAGSATTO AITBEGT ALDBOOD AITBEZGG NATRIJ ALDGGSAG AAFATI Z AMASOGGO AAGGGGGB RTGI 14 ANYZAZZG NAGBGS ANGBODI JAITBAGI ALGSTATO AATTDA AISSSAAA ANYSZZGG
45	410295	11922_2	KATOTOSO AATISTS AUTOTO WISSOT HGDTS AVEGTOR A4GRS17 ALCIYOU WITHOUT GED IRESS ACCUZES HTSSI0 NO1548 ACCUZOR HGGTOR ANUTSZZZ AUZIZOR I GESTSI WRSIZZ AATIOTOR ARODES HZZSES FEZERB MYRSOZZ ALCIGEOR AUGZZES FEZER I WOTU FAMIRBOZ I ALZIGIOS WRSITZ AUGIZOR I GESTSI WRSIZZ AATIOTOR ARODES HZZSES FEZERB MYRSOZZ ALCIGEOR AUGZZES FEZER I WOTU FAMIRBOZ I ALZIGIOS WRSITZ
20	414496	1,2833,1	WYSSIA MASISTA WIRBEZZI AM1485ZA AM1481ZA AM1911ZA AM544ZA AZKESZE AKZSITOZ AM1944ZA AKZINDA HARIYAI HEFIZBA AW1881ZZI WYSSIS AWZISTI BAZISHIZ AH1887ZI AKZKESOD KZI 468 AW77DATB WYSZZZ AKSITOZA AM513SI Z AH4444B W7781B AAGAKSB AKZROD WSSZZZI ARRATY AM8
	418019	180623_1	aazagis motood aazasso aazagii moodoo aagasta aatobot rabas aazotti aabassa arsogia aatasaba ristiso nagbo mosasa arrsos aassesa
55	424651	241581_1	ROGEST AUROPT RANGTEN I AACTSTRA LANGORD ALSEGIZ AACTST 18 F3 TSG ANTYSTYP HIGGIS AAGSTSG AACTSTIS AACTSGIS AAGSTSGI AAWSSCORS TAKERSGIS AAKSTSGIS TAKESGIST AATSGIST HIGGIS HIGGIS AAGSTOOB AAVSKIS WIDTS AATSGISTA AAATSGIS AAKTSGIS AAKTSGIS AAGSTSGIS SOOS
	436772	46501_1	AWSTSER JAZINSKI KOTORA MOGISO KODSOLO KODORA BEGITTY AADOSOG BE 169200 TATITB AWSMIZA BESCIAIS AALZ1893 AZSSZOJ TACH ARGASG9 AASSTOH MOGISOL JAZINIH RETYTTIA AWSTIAI AWSMICKAR AABOLOON ESTIFICIA
9	44931	628799	ANGESTI AAGTISTE ALGETTÄTZ. REGOOG AALGEGED TEIGT TEGSET AKSTISS KURGED BETOT ANGEAUT AAGSUCH ANGSGED DEZBEA ANVELIGES AALGEGES ANGERES BARBEBOOLARTIZK ALGESTE ALTIGGER AALGESSE AZTISTE FALLERI HAAGSICH ANGEGE ANGSSES ALGEST AALGEST AALGEGE
y	451573	875588_1 488021_1	ANY TOSSS ALIB ANY TOSS A WALKERS METGRITS ANY TSSSY AWAGETI KWAGGOZ AMAGAGO ANY TSSSOA ANTSSTOO

TABLE 24C:

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Play: Unique number corresponding to an Eca probeset
Ref. Sequence source. The 7 digit numbers in this column ere Genthank Identifier (GS) numbers. "Duntarn I. et al." rates to the publication entitled. The UNIA sequence of
Ref. and Committee of a (1999) Walter 807-818-455
Street. Indicates UNIA stand from white new were produced account RULPARS.
Street. Indicates Indicated positions of practical excess.

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Table 25A provides Unigenel ID, Unigenel TRig, Priey, and Exempter Accession for sequences in Table 26. The information in Table 25A is isnived by SEQ ID NO: is Table 26. 80

Tatha 25A: Pkey: Unique Ecs probaset identifies number

WO 02/102235

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Ex. Acor: Exampler Accession number, Gentuent accession number 1000. Underne number number 1000. Underne number 1000. Sequence identification number for sequences in Table 28 SEQ ID NO. Sequences in Table 28

'n					
	Play	Ex. Acc	0.00	Title	SEOID NO
	421478	Al683243	H. 97258	ESTs, Moderatory shrifter to \$29539 floor	SEQ ID NO. 34
2	4369B2	AB018305	Ha.5378	spondin 1, (1-spondin) extracellular mai	SEO ID NO: 5-6
2	122054	AW088542	Hs.97384	SRY (sex determining region Y)-box 17 (5)	SECTION OF 1-8
	10102	AW248508	Hs.279727	ESTs; homotogue of PEM-3 (Clora saviony	SEQ 10 NO: 11-12
	428579	NM_005756	Hs.184942	G protein-coupled receptor 64	SEO ID NO: 13-22
15	428227	AA321649	Hs.2248	small inductive cytokine subfamily B (Cy	SEO 10 NO: 23-24
2	451110 428187	AKRTIO	He 285570	PAR-0 Dolla (paradora) delectro o n Giombelo-market recentor 49	85-010 NO: 27-28
	(2,00)	NM 002497	Hs.153704	NIMA (never in milosis gene a)-related is	SEC 10 NO: 29:30
	(33159	AB035898	Hs. 150587	kinesh-like protein 2	SEO ID NO: 31-32
•	428427	WB6699	Hs.169840	TTK protein kinase	SEO ID NO: 33-34
07	12537	D49441	Hs.155981	тезорый	SEO ID NO: 35-39
	4.8508	AA084248	Hs.85339	G protein-coupled raceptor 39	SEO IO NO: 39-40
	126546	Al690321	Ha.203845	KCNK15 potasstum channel, eublamity K, m	SEQ ID NO: 41:42
	441344	A 1245671	7775	O-nyotoxyeypaetrine (seroantii) receptor CCC The domein middale 8	SECTION OF ALL
25	474620	AA101043	Hs. 151254	kalikesh 7 (dysmotyvale statum.com	SEO ID NO: 47-48
ì	12078	X69699	Hs.73149	paired box gene 8	SEO ID NO: 49-52
	409178	BE393948	Hs.50915	kathrein 6	SECTIONO: 53-54
	448243	AW369771		integrin, beta 8	SEO ID NO: 55-68
ć	426514	BE616633	Hs.170195	bone morphogenetic protein 7 (osteogenic	SEC 10 NO: 57-58
3	419452	U33636	Hs.90572	PTK7 protein tyrosine kinase 7	SEO ID NO: 59-60
	213	NW 006103	Hs.2719	HE4; epidldymis-specific, whey-acidic pr	6EQ 10 NO: 61-62
	413373	AI/33881	12,724.2	HANNEL (NO.E 2)-e1	000 ID NO. 05-04
	10007	013550	12.136348	perosum (USF-208)	SEC 10 NO. 65-66
35	133001	DE2/1200	D7170784	and caccinates augmented gracement	200 C C C C C C C C C C C C C C C C C C
ç	1007	1,503/	H3.1564	cartage digometric muniti protein (pae	550 D NO. 55-73
	700	ANDUMA	14.26303	hypothetical protein PLL 2416	860 D NO. 73 74
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40	426215	AW963419	Ha 155223	stantication 2	850 D NO. 75 BB
:	43004	AA464510	Hs.152812	ESTs	SEO ID NO: 81
	447033	AI357412	Hs.157601	ESTs	SEO ID NO: 82-87
	410418	031382	Hs.63325	transmembrane proteasa, serine 4	SEO ID NO: 88-89
,	411274	NN_002776	Hs.69423	katikrein 10	SEQ 10 NO: 90:01
<del>2</del>	\$226	AA315983	Ha. 105484	regenerating gans type IV	SEO ID NO: 92-93
	060	AB033025	Hs.50081	Hypothetical protein, XP_051850 (NAA119	SEQ 10 NO: 94:95
	52000	AK001666	HS.169095	SITUATION DE SALLY (SEE (DIOCODINIA)-HIND	SECTION OF SEST
	100	307777	11- 100000	Insulin-like growth factor 2 (comatomed)	SECTION SESS
Ş	17.	AW411425	MS. 180655	Series (Newsonian Marks 12)	
3	431878	PE010074	13.7.5023 Lie 27.1580	read along the samp, to span-sit (rankings	SEO ID NO. 104-105
	425465	1.18964	190	omplete three C inta	SEO ID NO: 108-107
	432938	127013	Hs.3132	steroldogenic acute regulatory protein	SEO IO NO: 108-109
;	421451	AA291377	Hs.50831	ESTs :	SEQ ID NO: 110-117
S	437478	AL390172	Hs.317432	branched chain aminoransierase 1, cytos	SEQ 10 NO: 118-119
		AL0335Z/	H8.9213/	L-myc-z protein (MYCLZ)	SECTIONO: 120-121
	408400	C700000	rs. 139033	paganany administration of the self-refundamental (KL)(S)	SECTIONO: 124-125
;	428450	NM_014791	Hs. 184339	KIAA0175 gene product	SEQ 10 NO: 126-127
9	438167	R28363	Hs.24286	chemoldne binding protein 2 (CCBP2), mRN	SEQ 10 NO: 128-129
	416530	U52801	Hs. 79361	kalifuein 6 (neurosin, zyme)	SEQ ID NO: 130-131
	1000	C14187	H8.157208	Intersect retailed homeobox protein AKX	SEC 10 NO: 132-133
	1177	NU OUETOD	He 72078	entrol of contributions and the field	SEO ID NO. 134,138
65	407792	AI077715	H,39384	outsine secreted found homologous to f	SEQ 10 NO: 139-140
	426093	AW594506	Hs. 104830	ESTs	SEQ ID NO; 141-144
	431630	NM_002204	Hs.265829	integrin, alpha 3 (antigen CO49C, alpha	
	121502	A-11(356	18.10503 14.7364	south carrier tamily 34 (sociam phospinal	SEC 10 NO: 149-150
70	1369	BE1844SS	Hs.251754	secretory leukocyte protesse Inhibitor (	SEO ID NO: 153-154
	436972	AA284678	Hs.25640	claudin 3	SEC ID NO: 155-156
	429504	XS9133 APD41038	Hs.204238 Hs 57771	Spocesin 2 (oncogene 24p3) (NGAL) trafibrein 11	SEO 10 NO: 157-158 SEO 10 NO: 159-150
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	AGTCATGAMA	TTTTATATGC	TTITATATCE AGAGAGAAA AGTTACCGAG ACAGAAACA AATCTAAGGG	AGTTACCOAG	ACAGAAAACA	AATCTAAGGG	1540
Š	MIN		TATEGOATTA AGCTGAGCAA GCAATTCTGG TOGAAAGTCA AACCTGTCAG	SCATTCTOO S	TODOMESTICA	AACCIGICAG	3600
Ē	SCHOOLOGIC		PAGGCTOTO GTCCTCCCAD ACATGCATAG	ACATGCATAG	GANTGGCCAC AGGITTACAC	AGGITTACAC	3660
É	<b>BOCTTCOO</b>	<b>OCMITATA</b>	GCACACCAGA	TTCAGGGAGA	CTGACCGACCA	AGGGATAGTG	3720
ž	PANAMOGACA			FOGGTCCATC AGCAGTTTTT	CTTCCTGCAT		3760
ş	MACTATTOT		CTITIATAGO	CCTTATTACT	OCTTANTOCA	AATGTGTACC	3840
ŧ	ATTOGTORDA	CACATACAAT	OCTCTGAATA	CACTACGAAT	TIGINITAM		3900
ž	PATTICCANA	TACAACATAG	TATAGTCCTG	AATATOTACT	TITACACAA GAGAGACTAT	GAGACTAT	3960
2	CANTAMA	CTCACTGGGT	CTTTCATOTC	TITAAGCTAA	GENAGROTTC	AGAAGGTTCT	4020
E	TITTATATI	<b>STCCTCCACC</b>	TCCATCATT	TCAATAAAG	ATAGGGCTTT	TOCTCCCTTO	4080
Ĕ	TCTTCGAGG	GACCATTATT	DACCATTAIT ACATCTCTGA ACTACCTTTG	ACTACCTTTG	TATCCAACAT GITTIMATC	<b>OTTITAMATO</b>	4140
E	TTAAATGAA		TTOCTITICTE CCANANAGO	CACATATAA	CACANTATAA AGAAACACAA GATITAATTA	GATTTAATTA	4200
E	TITTCHACT		TOGGGGGAAA AAAGTCCTCA		TOTAGAAGCA CCCACTITIG	CAATOTTGIT	4260
ŧ	TAAGCTATC	TATCTAACTC	TCAGCCCATG		ATARAGITEC TIMAGCIGGT	GATTCCTAAT	4320
3	CANGGACAAG	CCACCCTAGT	GTCTCATGTT	TOTATTTOOT	COCAOTTGGG	TACATTITAA	4380 .
ž	VICCIOAII	TTGGAGACTT	AAAACCAGGT	TAATOGCTAA	DALTOOOTAL	CATGACTETT	4440
E	TTCCATTGT	TATTTTOT	TTGCAATGGG	GAATTTAAA	GAATITIATAA GAAGCATCAA GICTCTITICI	OTCTCTTCT CT	4500
Š	PACCAAAGTC		GTTTATAGIT	CTTTTOOCTA	CTTTTOOCTA ACAAATCATT	TTGGAAATAA	4560
ğ	AGATTTTTA	CTACABAAAT					

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	Protein Acc	Protein Accession #r BAD10461	3AD10461				
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3	MRLSPAPLKL	MRISPAPLKL SRIPALLALA LPLAAALAPS DRILDKVPKS EGYCSRILRA OGTRREGYTE	LPLAAALAPS	DRILDKVPKS	ECYCSRILPA	<b>OCTRRECYTE</b>	ê
	PSLRVEGDPD	PSLRVECOPD PYRPOTSYRV TLGAAPPSYP ROFTLIALAR NRECOREEDH ACTPOIIDEE	TLBAAPPSYP	ROPTLIALRE	NREGIDKEEDH	AGTPOLIDEE	220
	ETOFMSNCPV	ETGEMSNICHV AVTRSTPRRR TRIGVPHIAP PAGTGCVILK ASLVOKRIIY FODEGSLTKK	TRIOVPWIAP	PAGTGCVILK	ASIVORRITY	PODEOSUTIKE	160
	LCEGOSTFOG	LCEGOSTFDG VTDKPILDCC ACGTAKYRLT PYGNWSBKTH PYDYPRRANH WSALIGGSHS	ACGTAKYRLT	PYGNMSEKTH	PYDYPRRAWH	WSALIGGENS	2
	<b>FOYYULNBYOO</b>	knyvlnbygg yasegvkgva blospvknez birqqsdbvl tvikakaqnp amqplinvraa	BLOSPVIORES	<b>EIRQOSDEVL</b>	TVIKAKAQNP	AMOPLINVRAA	ě
ಽ	PSAEPSVDRT	PRAEPBYDRT RHLASPLINA CPRPOMINGL BARDLCTKEC GWYGKVYGDL IPWDAGIDSG	CPEPDWAVGL	BAEDLCTXEC	GHVQKVVQDL	IPMDAGTDSG	š
	VTYESPNKPT	IPQEKIRPLT	IPQEKIRPLT SLOHPOSPPY DPEGGSITOV ARVVIERIAR KGEQCHIVPD	DPEGGSTTOV	ARVVIERIAR	KGEQCHIVPD	Ş
	NYDDIVADLA	NVDDIVADLA PEEKDEDDTP ETCIYSNWSP WSACSSSTCD KGKRNRQRML KAQLDLSVPC	ETCIYSNWSP	WSACSSSTCD	KGKRMRQRML	KAQLDLSVPC	9
	PDTQDFQPCM	Phygoropec GPGCSDEDGS TCTMSEWITH SPCSISCOMG MRSRERYVRQ	<b>TCTM9BW1TW</b>	SPCSISCOMO	MRSRERYVKO	PPEDGGVCTL	Š
;	PTESTERCTV	PTESTERCTV NEECSPSSCL MIGNOENDEC SATCONOMIX HHRMINMNPA DCSMCKAETS	MTEMBENDEC	SATCOMONIX	THRMI KMNPA	DGSMCKAET8	8
35	OVEKCHOPEC	DAEKOMPEC HTIPCLLSPM SEMEDCSVTC GROMRTRORM LKSLARLGDC NEDLEGVEKC	SEMEDCBVTC	GKGMRTRQRM	LKSLABLGDC	NEDLEGVERC	99
	MLPECPIDCE	MLPECPIDCE LIEMSQMSEC NKSCGKGHVI RTRMIQMEPQ PGGAPCPETV QRKKCRIRKC	NKSCGKGRVI	RTRMICMEDO	PGGAPCPETV	QRKKCRIRKC	22
	LRNPSIOKLR	LRNPSIOKLR WREARESRRS EQLKERSEGB OFPGCRMRPW TAMSECTKLC GGGIGERYMT	EDIKERSEGE	OFFICEMRPH	TANSECTICLE	GGGIQERYMT	5
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	Coding sequ	sequence: 2051449	Ξ				
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45	de la constante la	TACACCOCC	)	- Correction	AGACTAGACT		9
!	AGAACACOOG	0000000110	GGGCCGGCAG	ACCCGGGCAG	CCCCCCCCCCC	ATCTCAGTGC	120
	CTCATTCCCC	ACCCCCTCCC	CCGGGTCGGG	OCAGGCGGC	COTCCCCCCCC	AGGGTTGAGG	8
	GGAGCGGGC	AGGCCTGGAG	COCCATGAGC	<b>АВСССОВАТО</b>	COCCATACGC	CASTUACGAC	5
	CAGAGCCAGA	CCCAGAGGGC	actacceaea	OTTOATOCCC	DOCUCOCCC	CTOCCCCTGG	Š
2	GCCGAGTCGC	TCAGCCCCAT	COCOCACATO	AAGGTGAAGG	<b>OCCAGGCGCC</b>	GGCGAACAGC	360
	GGAGCACCGG	202000000	GOCCCAGCC	AAGGGCGAGT	CCCGTATCCG	GCGGCCGATG	420
	AACOCTTTCA	1001010000	TAAGGACGAG	COCMOCOC	TOCKSCAGG	GAATCCAGAC	=
	CTGCACACG	CCGACTTCAG	CAAGATGCTG	GGCAAGTCOT	GCAAOOCGCT	GACCCTOCCC	3
;	GAGAAGCGGC	CCTTCGTGGA	OGNOCCAGAG	COCCTCCCC	TOCAGGACAT	OCHOGACCAC	9
ŝ	CCCAACTACA	AGTACCGGCC	acaacaacac	AAGCAGGTGA	AGCOCCTGAA	GCGGGTGGAG	9
	GOCGCCTTCC	TGCACGCCT	COCTOACCC	CAGGCGGCCG	200000000	CCAGGGCGGC	ž
	OCCUTOCCA.	TOGACOCCT	GGGCCTCCAG	TTCCCCGAGC	AGGCTTCCC	0000000000	ĕ
	CCCCTGCTGC	CTCCGCACAT	GOGOOGCCAC	TACCOCCACT	GCCAGAGTCT	GOGCOCCCT	ž
,	CCCCTCCACG	<b>GCTACCCGTT</b>	BOCCACGCCC	GACACOTCCC	COCTOGACGO	COTOGACCCC	ĕ
3	GACCCOGCTT	TCTTCOCCOC	CCCGATGCCC	DOCUMENTOCC	0000000000	CACCTACAGC	9
	TACOCOCAGO	TCTCCCACTA	CGCTCGCCCC	CCGGAGCCTC	CCGCCCCTCC	CATOCACCCC	502
	COACTICOOCC	CAGAGCCCGC	0001000100	ATTCC0000CC	recroacee	ACCCAGGGCC	108
	CTTCACOTTO	_	GATGGGCTCG	_	0000000000	COGCTTCCAG	ž
;	ATGCAGCCGC	-	CCAGCACCAG	CACCAGCACC	ACCCCCCCGG	_	120
S	CCGTCCCCCC	CTCCOGAGGC	ACTOCCCTOC	CCGGACGGCA	COGACCCCAG	•	756
	QAGCTCCTCG	GOGAGGTOGA	CCCCACCCA	TITIONACAST	ATCTGCACT	COTTOTICCAAG	132
	CCTCMGATGG	_	CCAGGGGGCAT	_	TGAATCTCCC	COACAGCCAC	2
	00000CATTT	CCTCOOTGGT	<b>OTCCSACCC</b>	AGCTCCGCGG	TATATTACTO	CARCTATOCT	ž
ç	GACOTTOTGAC	-	1000000000	CCTGCAGGC	AGAAGCAGTG	TTACACACT	150
?	CCTOGAGGAG	•	ממקשמת	•	OTTOTTOCHO	•	126
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	1000000010	_	TAMATITOT	TCAGAGATTT	<b>OTTICOCACA</b>	<b>OTTOGATICI</b>	Ē
	CAMACCETA	•	CMGTTAACT	_	<b>GTGTCCCAAA</b>	ACADCTTOCT	174
75	CCATTICCTO	_	GATCAAAGAA	7	accretorin	TTTCAATCTT	ě
2	CTAAAAATA	MATCTCCAA	TCCTGAAAAA	AAAAAAAAA	AAAAAAAAAA	¥	

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	Protein Acc	Protein Accession #: NP_071899	IP_071899				
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	MSSPDACYAS	KESPDACYAS DOGSOTOSAL PAVNAGLOPC PWAESLSPIG DMKVKGEAPA NSGAPAGAAG	PAVMAGLOPC	PWAESLSPIG	DMKVXGEAPA	NEGAPAGAAG	9
	RAKGESRIRK	AKGESRIRR PHNAPHVWAK DERKALAQQN F	DERICALADON	POLITIVAELSK	POLIRVAELSK MLGKSWKALT LABKRPPVBE	LABICAPIVEE	120
	AERLEVORMO D	DHPNYKYRPR	RRKQVKRLKR	dhpatacarr rakquarlar vaxgplhgla bpqaalopb ogrvandglo	BPOAAALOPB	OGRVANDGLO	180
,	LOFFEQUEPA	DEPLICEPEND G	GHYRDCQ912	HYRDCQSLG APPLOGYPLP TPOTOPLOGY DPDPAFFAAP	TPOTOPLOGY	DPDPAPFAAP	540
2	<b>WPGDCPAAGT</b>	PROCESAGT YBYAQVEDYA GPPEPPAGPM HPRIGPEPAG PSIPGLIAPP BALHVYYGAM	GPPEPPAGPM	HPRLOPEPAG	PSIPCLLAPP	BALHVYYGAM	8

ОЗРФАССОКО РОМОРОНОНО НОНОНИРРОР ОДРВРРЕМЬ РОЖИСТИРВО РАЖЬКОВУОЯ ТЕРБОУЬНРУ СКРЕМСЬРУО СИБСТИНЬР ВКОАТВЯУИВ БАВВАУУЧСИ УРБУ Seq ID No. 10 Protein sequence Protein Accession #: Q9H8V3 S <del>수</del> S 

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WO 02/102235

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Seq ID NO: 153 DNA sequence Nucleic Acid Accession #: NM 003064.2 2

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Seq ID NO: 155 DNA sequence Nucleic Acid Accession #: NM\_001306.1 Coding sequence: 199..861 45 20

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sequences of accession numbers, and patent applications cited in this specification are herein. It is understood that the examples described above in no way serve to limit the true scope of this invention, but rather are presented for illustrative purposes. All publications, incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

PCT/US02/19297

## WHAT IS CLAIMED IS:

- A method of detecting an ovarian cancer-associated transcript in a cell
  - from a patient, the method comprising contacting a biological sample from the patient with a
- polynucleotide that selectively hybridizes to a sequence at least 80% identical to a sequence
- as shown in Tables 1-26.
- The method of claim 1, wherein the biological sample comprises
- isolated nucleic acids.
- The method of claim 2, wherein the nucleic acids are mRNA.
- The method of claim 2, further comprising the step of amplifying 4.
- nucleic acids before the step of contacting the biological sample with the polynucleotide.
- The method of claim 1, wherein the polynucleotide comprises a
- sequence as shown in Tables 1-26.
- The method of claim 1, wherein the polynucleotide is immobilized on

a solid surface.

- The method of claim 1, wherein the patient is undergoing a therapeutic
- regimen to treat ovarian cancer
- The method of claim 1, wherein the patient is suspected of having ∞
- ovarian cancer.
- An isolated nucleic acid molecule consisting of a polynucleotide ď
- sequence as shown in Tables 1-26.
- The nucleic acid molecule of claim 9, which is labeled. <u>6</u>
- An expression vector comprising the nucleic acid of claim 9. Ξ:
- A host cell comprising the expression vector of claim 11. 15.
- An isolated polypeptide which is encoded by a nucleic acid molecule 13.
- having polynucleotide sequence as shown in Tables 1-26.

WO 02/102235

PCT/US02/19297

- An antibody that specifically binds a polypeptide of claim 13. 4.
- The antibody of claim 14, further conjugated to an effector component. 15.
- The antibody of claim 15, wherein the effector component is a 16.
- fluorescent label.
- The antibody of claim 15, wherein the effector component is a 17.
- radioisotope or a cytotoxic chemical.
- The antibody of claim 15, which is an antibody fragment. 18.
- The antibody of claim 15, which is a humanized antibody 6
- A method of detecting an ovarian cancer cell in a biological sample 23
- from a patient, the method comprising contacting the biological sample with an antibody of
- claim 14.
- The method of claim 20, wherein the antibody is further conjugated to
- an effector component.
- The method of claim 21, wherein the effector component is a 22
- fluorescent label.
- A method for identifying a compound that modulates an ovarian
- cancer-associated polypeptide, the method comprising the steps of:
- (i) contacting the compound with an ovarian cancer-associated polypeptide,
- the polypeptide encoded by a polynucleotide that selectively hybridizes to a sequence at least
- 80% identical to a sequence as shown in Tables 1-26; and
- (ii) determining the functional effect of the compound upon the polypeptide.
- A drug screening assay comprising the steps of 24.
- (i) administering a test compound to a mammal having ovarian cancer or a cell
- isolated therefrom;
- (ii) comparing the level of gene expression of a polynucleotide that selectively
- hybridizes to a sequence at least 80% identical to a sequence as shown in Tables 1-26 in a

W0 02/102235

PCT/US02/19297

treated cell or mammal with the level of gene expression of the polynucleotide in a control

cell or mammal, wherein a test compound that modulates the level of expression of the

polynucleotide is a candidate for the treatment of ovarian cancer.

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